REVIEW
SILURIAN-DEVONIAN ORIGINS OF FERNS AND LYCOPHYTES - WHAT WE KNOW, WHAT WE NEED TO FIND OUT

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ABSTRACT

This represents a synopsis of current knowledge of the Siluro-Devonian fossil record concerning evolution of lycophytes and ferns. This is the time period when several taxa or lineages at different grades of organisation existed that may be informative about the origins of these groups or structures typical of these groups. Considerable new data, including earlier first appearances of lineages and plant structures, new data about Siluro-Devonian lycopsids or basal euphyllophytes, and new whole plant reconstructions of small to tree-size plants in both lineages, have been published in recent years. It is not possible to be completely comprehensive, but the taxa discussed are either central to established ideas, or provide new information in relation to phylogenetic relationships and evolutionary trends. It remains difficult to trace the phylogenetic relationships of early plants relative to extant lineages. New data are reviewed which may be important in reassessing homology of characters and/or hypotheses of such relationships or in determining which taxa to exclude. Including fossils in estimates of relationships of these major lineages of plants will provide a more accurate and comprehensive understanding of the past history of seedless vascular plants.

INTRODUCTION

A consensus classification of extant seedless vascular plants by PPG1 (2016), reflecting molecular and some morphological phylogenies (Smith et al., 2006b; Kenrick & Crane, 1997; Pryer et al., 2001; 2009; Schneider et al., 2009; and others) recognises Lycopodiopsida (with three families and collectively referred to as lycophytes) and a grade "euphyllophytes" which consists of two clades - seed plants and Polypodiopsida (Figure 1). The latter includes four subclasses, Equisetidae, Ophioglossidae, Marattiidae and Polypodiidae- all referred to as ferns.

Lycopodiopsida appears to represent a monophyletic group defined by both molecular and morphological characters that diverged at least in the mid-Silurian if not earlier (Kenrick & Crane, 1997; Nickrent et al., 2000; Renzaglia et al. 2000; Pryer et al., 2001; 2004; Qiu et al., 2007; Li et al., 2014) but see Hao and Xue (2013) for a different interpretation. Relationships of several putative stem lycophytes such as some *Cooksonia pertoni, Aberlemnia (formerly Cooksonia) caledonica*, *Renalia* and several others, some within more derived extinct clades where critical characters are missing, are poorly resolved (Kenrick & Crane, 1997; Gensel & Berry, 2001; Hao & Xue, 2013). Additional data and more whole plant reconstructions of several Upper Silurian or Lower Devonian polysporangiophytes are needed to address these issues.

The term "fern" or "fern-like" now encompasses the majority of extant and extinct non-lycophytic seed-free vascular plants and several extinct "stem" euphyllophytes (trimerophytes), those with "peripheral permanent protoxylems" such as cladoxylopsids, iridopterids, and in some cases, rhacophytes and stauropterids (Kenrick & Crane, 1997; Rothwell & Stockey, 2008; Rothwell & Nixon, 2006; Berry & Stein 2000; Berry & Wang 2006). This results in a broad view of what represents a "fern". This is tied up in considerations of evolution of the so-called megaphyllous leaf via modification of branch systems. However it is clear such leaves evolved many times and possibly from different parts of lateral branch systems. Furthermore, using criteria such as presence/absence of a leaf gap and simple *vs* branched venation to distinguish microphyll *vs* megaphyll is not clear-cut. For a more complete discussion of these issues, see Tomescu (2009) and later sections of this paper. The term megaphyll is intentionally not used in this paper.

Fossils were intentionally excluded from the PPG1 classification, because "the phylogenetic affinities of most extinct plants are rather unclear" (p.565). While a practical starting point for names, it, along with molecular- derived phylogenies of only extant forms, implies misleading relationships by ignoring some of the major discoveries in the fossil record of the past several decades. For example, stating that ferns are the closest living relative to seed plants is correct when only living plants are considered, but ignores the existence of extinct plants with unique combinations of characters representing one or more now-extinct lineages, notably progymnosperms, but also others, indicating a more distant relationship between seed plants and ferns. While molecular-based phylogenies may suggest a close relationship among the extant subclasses, incorporation of fossil and morphological data (Figure 2) suggest they are not monophyletic (Rothwell & Nixon, 2006).

The fossil record, while still incomplete, presently suggests each Polypodiopsida subclass (PPG1 2016) differs in time of first appearance and possibly in origin. First appearances of undoubted extinct members of each clade are as follows: equisetophytes -the Late Devonian, filicaleans (extinct Polypodiidae) - Early Carboniferous, Marattiidae -Late Carboniferous, and Ophioglossidae -Cenozoic. Thus time gaps exist between the later appearing taxa/lineages and putative early representatives of those clades. Finding morphologically based synapomorphies to unite extant and extinct putative progenitor

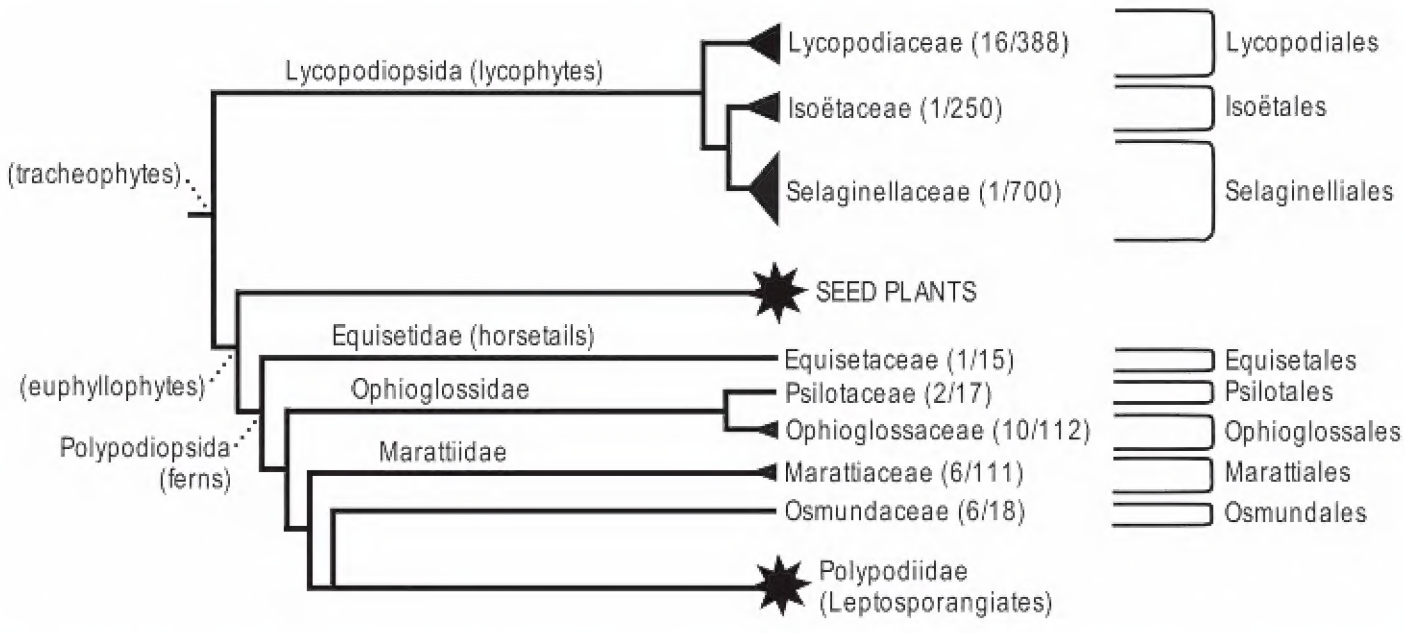

Figure 1. A simplified phylogeny of ferns and lycophytes, showing only the major lineages, modified from Figure 1 of the Pteridophyte Phylogeny Group 1 (PPG1) study.

or sister lineages is difficult. Characters that have been commonly employed among the Devonian age euphyllophytes are 1) branching systems, from which leaves are believed to have evolved and part or all of which are often interpreted as "leaf-equivalents", 2) stelar anatomy and 3) protoxylem location. Sometimes interpretations are retrospective, with extant leaf morphologies influencing ideas of what constitutes a leaf-equivalent. Not everyone agrees on which part or parts of a branching system constitutes a leaf-equivalent in each lineage, and lastly, for some, all leaves are considered homologous (ignoring the fossil record) rather than evaluating the fossil plants within the context of their time and environment (Berry & Stein, 2000) and taking a more "bottom-up outlook" (Rothwell & Stockey, 2008) of extinct plants relative to extant ones. Recently acquired fossil data and whole- plant reconstructions, coupled with new developmental information or tests of certain character distributions, may improve assessment of homology of structures and character state transformations and ultimately phylogenetic relationships (Tomescu, 2009; Corvez et al., 2012 and authors cited therein). Additional work on understanding variation in anatomy also is needed. As our understanding of certain plant types improve, the fossil record may also indicate which possible progenitor candidates to preclude.

WHAT DOES THE FOSSIL RECORD CURRENTLY TELL US?

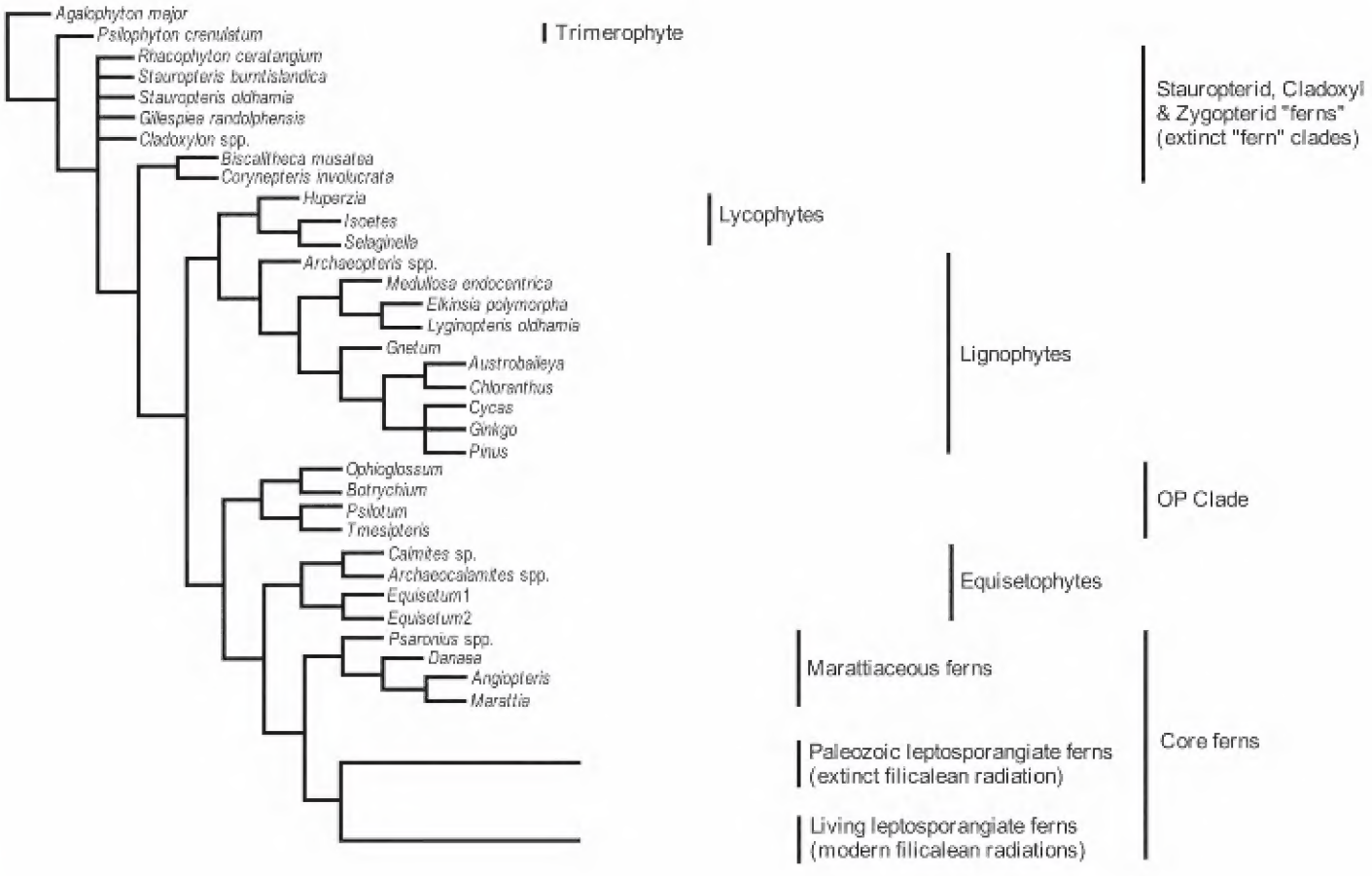

Figure 2. From Rothwell and Nixon, 2006 showing a tree based on a combination of fossil and extant plant morphological and molecular characters. Inclusion of fossils causes changes in postulated relationships of so-called "fern-like plants" relative to filicalean (=Polypodiidae) ferns. Terminology is that used by Rothwell and Nixon (2006). Lignophytes refer to progymnosperms and seed plants. OP= ophioglossid ferns and psilotophytes. Palaeozoic leptosporangiate ferns include taxa such as *Metaclepsydropsis*, *Ankyropteris, Psalixochlaena, Botryopteris*, and others.

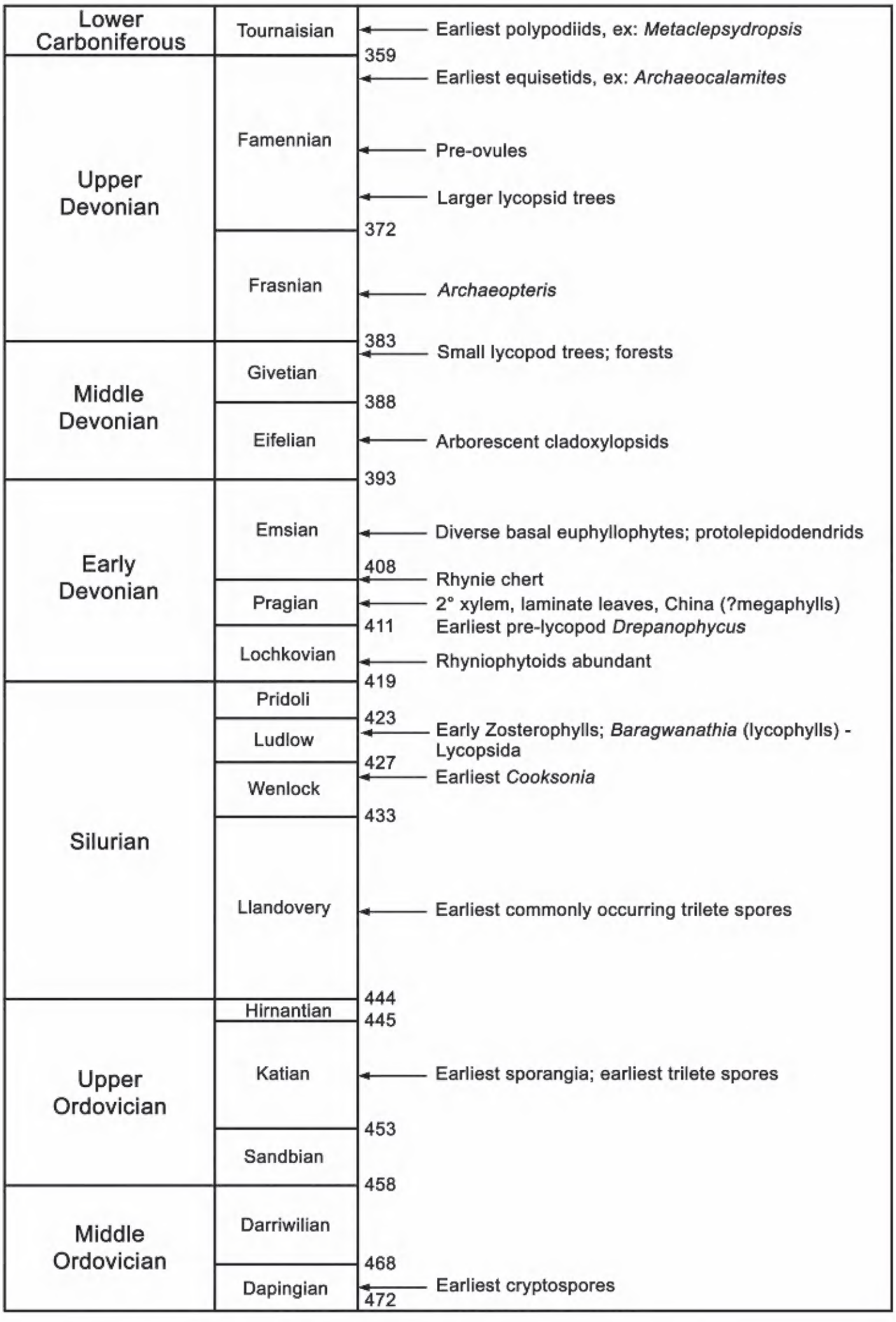

Figure 3. A stratigraphic chart showing major events in plant evolution or first appearances of certain plant groups or plant structures, spanning Ordovician to Tournaisian.

GENERALITIES

1) Everything happened earlier (Figure 3): Dispersed cryptospores and trilete spores provide evidence for Kingdom Plantae in the early Middle Ordovician to late Middle Ordovician respectively (Rubenstein et al., 2014; Steemans et al., 2009; 2010; Wellman et al., 2008; 2015; Kenrick et al., 2012; Edwards et al., 2014, Edwards & Kenrick, 2015, and references cited therein). Cryptospore types and diversity increase until the earliest Devonian, trilete spores diversify from Silurian on. Earliest forms occur in Gondwanan regions, suggesting radiation of cryptospore producers and basal polysporangiates from Gondwanan microcontinents towards Laurussia (Wellman et al., 2015; Steemans et al., 2010). The absence of megafossils during this time span may partly reflect the lack of known terrestrial sediments in the Ordovician and Silurian (Kenrick et al., 2012; Wellman et al., 2015). Some tiny Upper Silurian to Lower Devonian plants produce cryptospores, termed cryptophytes (Edwards et al., 2014), and others produce trilete spores, some possibly representing precursors of a bryophyte or polysporangiophyte grade (Edwards et al., 2014). Unfortunately, only some dispersed spores can be tied to parent plants, which limits establishing evolutionary/phylogenetic links between cryptophyte, bryophyte or polysporangiate lineages, and thus to either lycophytes or fern-like precursors (Edwards et al., 2014).

2) Innovations: The Silurian to Middle Devonian vascular plant diversification demonstrates a greater increase in plant diversity, size and complexity than previously known. Silurian to Early Carboniferous taxa exhibit a trend from considerable morphological/anatomical plasticity to more predictable body plans or architecture in younger, more derived forms (Bateman et al., 1998). Recent research suggests a transition from apparently rhizomatous forms to upright trees by the Middle to Late Devonian among lycopsids and euphyllophytes (Figure 3), tree size ranging from 3 to at least 8m tall and of varying trunk diameters (4 to ~20 cm wide). Some formed new vegetation associations, most notably early forests. In euphyllophytes, no laminate leaves and no, or limited, secondary tissues exist (thus having alternative means of mechanical support) (Stein et al., 2007; 2012; Giesen & Berry, 2013; Berry & Marshall, 2015). Archaeopteridalean forests of large trees with abundant secondary tissues dominated some regions in the Late Devonian. Evidence of early evolution of leaves and roots occurs from Late Silurian on, as discussed in greater detail later.

Another major innovation, aligned xylem meeting criteria for secondary xylem and interpreted as an early type of vascular cambium, is demonstrated by the Pragian *Amoricaphyton* (Gerrienne et al., 2011; Strullu-Derrien et al., 2013; 2014; Gerrienne & Gensel, 2015) and Emsian plants from eastern Canada (Gerrienne et al., 2011; Hoffman & Tomescu, 2013; Gensel in prep.). It is unknown if these possessed uni- or bifacial cambia. That, and lack of fertile remains, renders affinities and potential relevance as to how vascular cambia evolved or to fern and lycophyte vs. lignophyte evolution unclear. Uni- and bifacial vascular cambia originated more than once, as they are known in several extinct lineages from Middle Devonian on, including lycopsids (uni), progymnosperms (bi), early seed plants (bi), and possibly cladoxyls (unclear if secondary xylem or primary, aligned metaxylem).

Heterospory is established by the Middle Devonian in lycopsids and some euphyllophytes (the incipiently heterosporous Lower Devonian *Chaleuria*; the Upper Devonian archaeopterids and some Lower Carboniferous stauropterids). The Middle Devonian putative ovule precursor, *Runcaria* (Gerrienne et al., 2004) and numerous pre-

ovules of Late Devonian (Famennian) age indicate an initial radiation of early seed plants at nearly the same time as heterosporous euphyllophytes occurred (Prestianni & Gerrienne, 2010), but prior to appearance of all fern clades except Equisitidae.

LYCOPHYTE EVOLUTION

The presence of Late Wenlock *Cooksonia sp.* from Avalonia (Edwards & Feehan, 1980), and the Upper Silurian (Ludlow) occurrences of several zosterophylls, putative stem lycophytes, and the lycopsid *Baragwanathia* from Bathurst Island, Canada and/or Australia (Kotyk et al., 2004; Rickards, 2000) indicate several grades and clades of rhizomatous, homosporous lycophytes at that time and an early origin for lycophytes. Zosterophylls, stem lycopsids, pre-lycopsids and lycopsids occur widely in the Early Devonian. Strobilate probable zosterophylls such as *Distichophytum* and plants similar to *Bathurstia* first appear in the Late Silurian and similar forms are diverse in the Early Devonian.

Zosterophyllopsids are leafless but exhibit similar anatomy and sporangial morphology and dehiscence as lycopsids. *Kaulangiophyton,* lacking vascularized leaves, *Asteroxylon* with transfusion tissue in "leaves" (Hueber, 1992), and fertile *Drepanophycus* spp. all lack association of sporangia with leaves, and sometimes are considered pre-lycopsids (Gensel & Andrews, 1984; Gensel & Berry, 2001). Leaves of differing morphology occur in the Siluro-Devonian *Baragwanathia*, in the pre-lycopsid *Drepanophycus*, the putative lycopsid *Zhenglia* (Hao et al., 2006) and in some Pragian taxa of uncertain affinity from China such as *Adoketophyton* (Li & Edwards, 1992; Hao et al., 2003). Mid-Upper Devonian and younger lycopsids exhibit mostly laminate univeined lycophylls, some of which are much-divided (Protolepidodendrales) or have laciniate to fimbriate margins (e.g. *Longostachys, Wuxia*). Ideas about how lycophylls may have evolved vary but are unresolved, ranging from change via progressive vascularization of enations, sterilization of sporangia (Crane & Kenrick, 1997), or by reduction of branch systems (reviewed in Tomescu, 2009).

Growth habit among these basal groups (stem taxa, zosterophylls, pre-lycophytes, early lycopsids) is either rhizomatous or tufted (Gensel et al., 2001; Raven & Edwards, 2001; Gensel & Berry, 2001; Hao et al., 2010). Rooting structures appear to have arisen more than once, ranging from downward trending axes (some *Zosterophyllum* spp. of earliest to early Devonian age), to part of a bifurcation of a rhizome (H or K branching), with one half being stem-like and bearing enations or leaves, the other portion lacking those structures and appearing root-like (*Bathurstia, Drepanophycus* - Gensel et al., 2001). An un-named lycopsid from the Lower Devonian of Wyoming differs in that the initial K-branch produces a "root-bearing axis", from which dichotomizing root-like structures depart (Matsunaga & Tomescu, 2016). These authors suggest rooting structures in lycophytes are *de novo* structures rather than modified stems. Hetherington and Dolan (2017) note commonalities among lycopsid root morphology as well as disparities, recognizing multiple types of structures that bear the branched roots. None of the early forms are anatomically preserved, thus features that distinguish roots from stems (root cap, root hairs, etc) in younger plants are lacking (Raven & Edwards, 2001; Gensel et al., 2001; Kenrick & Strullu-Derrien, 2014).

The order Protolepidodendrales, plants characterized by much divided or hastate leaves, epiphyllous oval to possibly round sporangia, and faintly to deeply lobed protosteles, is established by the Emsian (*Leclercqia* spp.) and taxa are much more diverse and widespread in the Middle and early Late Devonian (Gensel & Berry, 2001).

Most specimens may represent more distal or aerial branches and presumably were rhizomatous or in one case, climbing (*L. uncinata* Xu et al., 2011). Oval to elongate sporangia attach to the upper surface of sporophylls identical to vegetative leaves. *Leclercqia complexa* from the Middle Devonian Gilboa, NY locality is known to be ligulate; *L. complexa, L.* cf. *complexa* and *L. uncinata* yield spores showing them to be homosporous (Grierson & Bonamo, 1979; Richardson et al., 1993; Gensel & Kasper, 2005; Gensel & Albright, 2006; Wellman et al., 2009; Xu et al., 2011). Spores and ligules are unknown for other genera. Rooting structures, which might aid in better understanding of relationships, are unknown in all taxa. These plants are often considered to be putative precursors to the ligulate heterosporous clades Selaginellales and Isoetales, but better understanding of all plant parts would allow a more precise assessment of their relationship to other extinct and extant clades. As noted below, additional putatively transitional forms exist, although many are incompletely preserved.

A major change in growth habit and reproduction occurred in the late Mid Devonian, as shown by the bipolar/pseudobipolar, approximately half metre tall tree-like taxa *Longostachys* and *Chamaedendron* (Cai & Chen, 1996; Schweitzer & Li, 1996), both of uncertain affinity, in China. *In situ* (and some isolated) lycopsid stems from the upper Middle/lower Upper Devonian of Svalbard, consisting of approximately 1m tall, incomplete trunk pieces, and identified as *Protolepidodendropsis pulchra*, are interpreted as a paleotropical forest (present in a low paleolatitude) by Berry and Marshall (2015). *Sublepidodendron,* especially *S. songziense* Wang et al. 2003*,* from the Late Devonian of China, was at least a metre tall. A smaller, very slender, upright and unbranched Late Devonian lycopsid, *Clevelandodendron ohioensis* (Chitaly & Pigg, 1996), exhibits a lobed/branched base and a bisporangiate strobilus reminiscent of some lepidodendrids. Wider stemmed lycopsids such as *Cyclostigma, Lepidosigillaria*, and the unidentified tree lycopsid from Gilboa, NY (Haughton, 1859; Chaloner, 1968; Kräusel & Weyland, 1949; Stein et al., 2012) also occur in late Mid to early Late Devonian in Laurussia. These taxa not only differ in size (length, width), but also in branching (presence/absence, type), leaf base patterns, leaves, rooting and reproductive structures. They also clearly demonstrate the existence of upright lycopsids of varying heights, diameters, and branching architecture by Middle to Late Devonian time, coexisting with the presumably rhizomatous protolepidodendrids and presaging growth habits and reproductive strategies present among the abundant Carboniferous and younger lycopsid lineages. Various isolated anatomically preserved stem remains, when viewed in comparison to anatomy in more extensively preserved plants, also show differences in some features, not yet well understood. Secondary tissues occur in lycopsids from the Middle Devonian. Tracheid wall pitting among different taxa varies from one or more of the following pitting types: scalariform, pitted, circular bordered pitted, scalariform with fibrils connecting the bars or pitlet sheets. Much remains to be learned about its possible taxonomic implications.

Different types of stem bases with rooting structures occur among these taxa. *Longostachys* and *Chamaedendron* stems (and possibly *Clevelandodendron*) terminate in a series of downward trending, bifurcating root-like structures, but details are poorly understood. The two major rooting structures of arborescent isoetalean lycopsids dominant in the Carboniferous occur by the Middle Devonian. The earliest records of cormose lycopsid bases may be those recorded by Xu and Wang (2016) from the late Middle-Late Devonian Hujiersite Fm, China and the bases of the more extensively preserved *Protolepidodendropsis pulchra*, of similar age from Svalbard (Berry &

Marshall, 2015). Late Devonian *Leptophloem* and *Otzinachsonia* also exhibit cormose bases. A more branched, stigmarian type rooting system (Wang et al., 2003) occurs in *Sublepidodendron* spp from China. Laurussian forms are less easy to interpret. Pigg (2001) suggests that cormose and stigmarian forms represent variations of a single rooting type, perhaps being influenced by ecological factors.

Some of these Devonian lycopsid stems or branches bear sporangia aggregated into strobili, ranging from mono- to bisporangiate (*Longostachys, Chamaedendron, Sublepidodendron, Cyclostigma*), while others apparently have alternating fertile and vegetative zones (*Monilostrobus* Wang & Berry, 2003*; Wuxia* Berry et al., 2003), as also occurs in younger tree lycopsids. The Upper Devonian *Wuxia* is interesting in that megasporangiate "cones/fertile zones" occur just below branching points of axes but presumed microsporangiate ones are terminal (Berry et al., 2003). *Hoxtolgaya robusta* Xu et al. (2012), Middle Devonian of China, is interpreted as arborescent based on 9cm wide stems of unknown length, and exhibits sporangia yielding spores of one size, interpreted as homospory, but preservaton is too incomplete to determine with certainty. In strobili or fertile zones, sporophylls are either identical to vegetative leaves, or modified with a broad fimbriate to spiny base and distal lamina, as in the Middle to Upper Devonian small tree-like lycopsids such as *Longostachys* (Cai & Chen, 1996), *Chamaedendron* (Schweitzer & Li, 1996) or *Wuxia* (and others) (Berry et al., 2003).

Berry et al. (2003) note that variation in fertile regions/structures and in stem anatomical features occurs earlier and more broadly in China than Laurussia, suggesting that perhaps China may have been an important area of early evolutionary innovation in clade(s) from which the major lineages of arborescent, heterosporous lycopsids of the Carboniferous and later arose. More whole plant reconstructions of several of these lycopsids are needed, and particularly, clarification of the organisation of basal regions, presence/absence of branching, anatomical features, and type of reproductive structures among individual plants, in order to better understand their relationships to both earlier and later occurring lineages to resolve relationships of these several plants and other taxa all considered as part of the isoetalean clade.

The record of herbaceous, homosporous lycopsids (e.g. Lycopodiaceae) is far less well known in the Devonian and Carboniferous. *Lycopodites oosensis* from the Late Devonian of Germany (Kräusel & Weyland, 1937) may represent one, but should be restudied. The Lower Carboniferous herbaceous form *Hestia* possesses too few characters to assess affinities (Bateman et al., 2007) but might represent an eligulate form. Younger fossils have been assigned to the genus *Lycopodites* but it is difficult to determine the affinities of these leafy stems; one has been found to represent conifer twigs, others exhibit a mix of lycopodiacean and sellaginellacean characters but are usually allied to Selaginellaceae (Skog & Hill, 1992; Thomas, 1992). Rowe (1988) identified a rhizomatous plant from the late Early Carboniferous Drybrook Sandstone as an early Selaginallalean, *Selaginellites resimus,* but little is known about early evolution of Selaginellales.

Several questions concerning early lycophyte evolution remain or are only partially answered by recent data. The paraphyly of some *Zosterophyllum* species and stem lycophytes remains unresolved despite new data. Much remains to be clarified about overall construction and relationships of the numerous Middle-Late Devonian protolepidodendraleans and small tree lycopsids, as discussed above, including how many times have structures such as strobili, ligules, rooting structures, and leaf abscission arisen and in what combinations? How do they relate to later-occurring lineages? And

again, questions remain about the early history of herbaceous, eligulate, homosporous Lycopodiaceae and ligulate, heterosporous Selaginellaceae through much of the Devonian and Carboniferous and even early Mesozoic.

EUPHYLLOPHYTE EVOLUTION

Extinct Devonian plants included in the Euphyllophyta mostly exhibit much branched aerial axes, some terminated by mostly fusiform sporangia. Anatomy consists of various protosteles (haplo-, actino-, dissected). Pertinent to fern evolution are stem euphyllophytes, such as *Psilophyton* and *Pertica*, the predominantly Mid-Upper Devonian Cladoxylopsida (=Pseudosporochnales [or Cladoxylales] + Iridopteridales), the enigmatic, but putative iridopterid *Rhacophyton*, and possibly Stauropteridaleans. The latter groups have been considered by many authors to be derived from a trimerophyte ancestor, and possible fern-like or fern precursors. Reasons vary; some derive from cladistic studies, others from older comparative studies. While lacking laminate leaves, either the whole or part of their lateral branch systems (LBS) are interpreted as showing possible stage(s) in the evolution of a leaf, as mentioned earlier (Kenrick & Crane, 1997; Gensel et al., 2001; Boyce & Knoll, 2002; Sanders et al., 2007; Tomescu, 2008; Galtier, 2010; Corvez et al., 2012). These occurred simultaneously but at different rates in different lineages (Galtier, 2010), and are not homologous within ferns or between "ferns" and seed plants. Secondly, some of the above taxa exhibit peripherally located protoxylem in various types of actino- or dissected protosteles (where anatomy is known) that differs from that present in aneurophytalean progymnosperms and early seed plants. The latter, and some basal euphyllophytes, exhibit radiate protoxylem (Beck & Stein, 1993), in which actinosteles or variants thereof have a central protoxylem, which appears to extend out each arm of the stele to produce lateral traces. These are considered mesarch but this depends on whether the protoxylem at the tips of arms persist, a feature presently not well understood. Anatomy of this type is typical of aneurophytalean progymnosperms, possibly some of the larger (*Pertica*–grade) trimerophytes, stenokolealeans, and other plants of uncertain affinity. However, it remains unclear if these two protoxylem patterns represent a good indicator of phylogenetic relationships. For example, Scheckler et al. (2006), in a re-study of *Langoxylon* Stockmans, a taxon of uncertain affinity, point out that *Langoxylon* stems possess a mixture of these two characters. *Pseudosporochnus hueberi* lacks a central protoxylem, but exhibits peripheral protoxylems plus several separate protoxylems along the midplane of an arm (Stein & Hueber, 1989), thus not all early euphyllophytes can be so easily categorized.

The term "peripheral loop", initially referring to an anatomical feature of Carboniferous zygopteridalean petioles, has been used to describe protoxylem features in many Devonian plants. As originally defined, a peripheral loop consists of a central rod of parenchyma surrounded by protoxylem (Leclercq, 1970) located near the margin of an elongated vascular bundle. Similar structures are termed the same in stems of Devonian taxa. Several studies show that most of the latter represent protoxylem lacunae, resulting from break-down of thinner walled protoxylem during development or represent a different organisation of protoxylem with associated parenchyma (Scheckler, 1974; Scheckler et al., 2006; Stein, 1982). This suggests that the presence of a so-called peripheral loop should be carefully evaluated in any Devonian plant, and is not phylogenetically useful. Furthermore, care should be taken in use of the term "clepsydroid" and in any phylogenetic implications that might follow, because strap-

shaped strands in main axes or lateral branch traces in Devonian plants expand at their ends in preparation for producing the next higher order traces, thus appearing similar to the dumbbell-shaped petiole trace so termed among some early ferns.

The earliest putative euphyllophyte laminate leaf may date from the Pragian *Eophyllophyton* (Hao, 1988; Hao & Beck, 1993), considered basal and sister to other euphyllophytes by Hao and Xue (2013) but considered of uncertain affinity by this author. While laminate leaves are absent in most other coeval taxa, they occur in the Early to late Devonian *Platyphyllum* (=*Flabellifolium*?) and the Late Devonian *Ellesmeris* (Hill et al., 1997), enigmatic *Shougangia bella* (Wang DM et al., 2015), archaeopteridaleans and early seed plants, appearing earlier in time in lignophytes than in filicales (Galtier, 2010). Clearly, resolution of what constitutes homologous structures in LBSs among these different much- branched plants, and more documentation of the first appearance of laminae, would address remaining questions concerning number of times and modes of leaf evolution in these groups and non-homology of so-called megaphylls in these groups.

CLADOXYLS, IRIDOPTERIDS AND RHACOPHYTES- CALLED "FERN-LIKE" BUT?

Starting in the Mid-Eifelian and extending through the late Devonian, several taxa referable to Cladoxylopsids s.l. (e.g. including iridopterids, Berry & Stein, 2000) and rhacophytes are part of a more complex vegetation, some of the former representing small to large trees recently described as major components of in situ Middle to early Upper Devonian forests in the USA and Germany (Stein et al., 2007; 2012). Concomitantly, major revision of taxon concepts for *Pseudosporochnus* (Berry & Fairon-Demaret, 1997; 2002), *Calamophyton* (now includes *Duisbergia, Cladoxylon*, and most species of *Hyenia* - Fairon-Demaret & Berry, 2000; Berry, 2005; Giesen & Berry, 2013) and *Eospermatopteris/Wattieza* (Stein et al., 2007; 2012) has resulted in critical new information about the architecture of these leafless trees.

Pseudosporochnaleans

Pseudosporochnus trees (Figure 4) produce rows of branches that abscise at their base; the branches are digitately divided and produce higher-order, divided laterals that are three-dimensional to subopposite and alternate, final divisions being vegetative and non-laminate or fertile (Berry & Fairon-Demaret, 2002). *Calamophyton* is a smaller tree, with a swollen base, stems enlarging near apex and bearing vertical rows of lateral branch systems that then divide digitately (Figure 5), and produce higher order helically arranged dichotomizing vegetative or fertile non-laminate units. Older branch systems break off above their base, leaving the *Duisbergia* type morphology (Giesen & Berry, 2013). *Eospermatopteris* is larger, with a swollen base, a wide stem, and crown of digitately divided branch systems of the *Wattieza* type (Stein et al., 2007; 2012), abscising at their base. Terminal fusiform sporangia are known for all of these, being either recurved (*Calamophyton*, *Wattieza*) or erect (*Pseudosporochnus*). These apparently represent the earliest tree architectural types among euphyllophytes, being similar to some palms, cycads and tree ferns (Stein et al., 2007) in stem and root structure.

Where anatomy is known in the pseudosporochnaleans, it varies from being a multistranded, anastomosing stele, producing similar but smaller multi-stranded steles in lateral branches, as in *Calamophyton* or *Pseudosporochnus* (Figure 6), to being comprised of individual radially oriented strands near the periphery of the stem adjacent

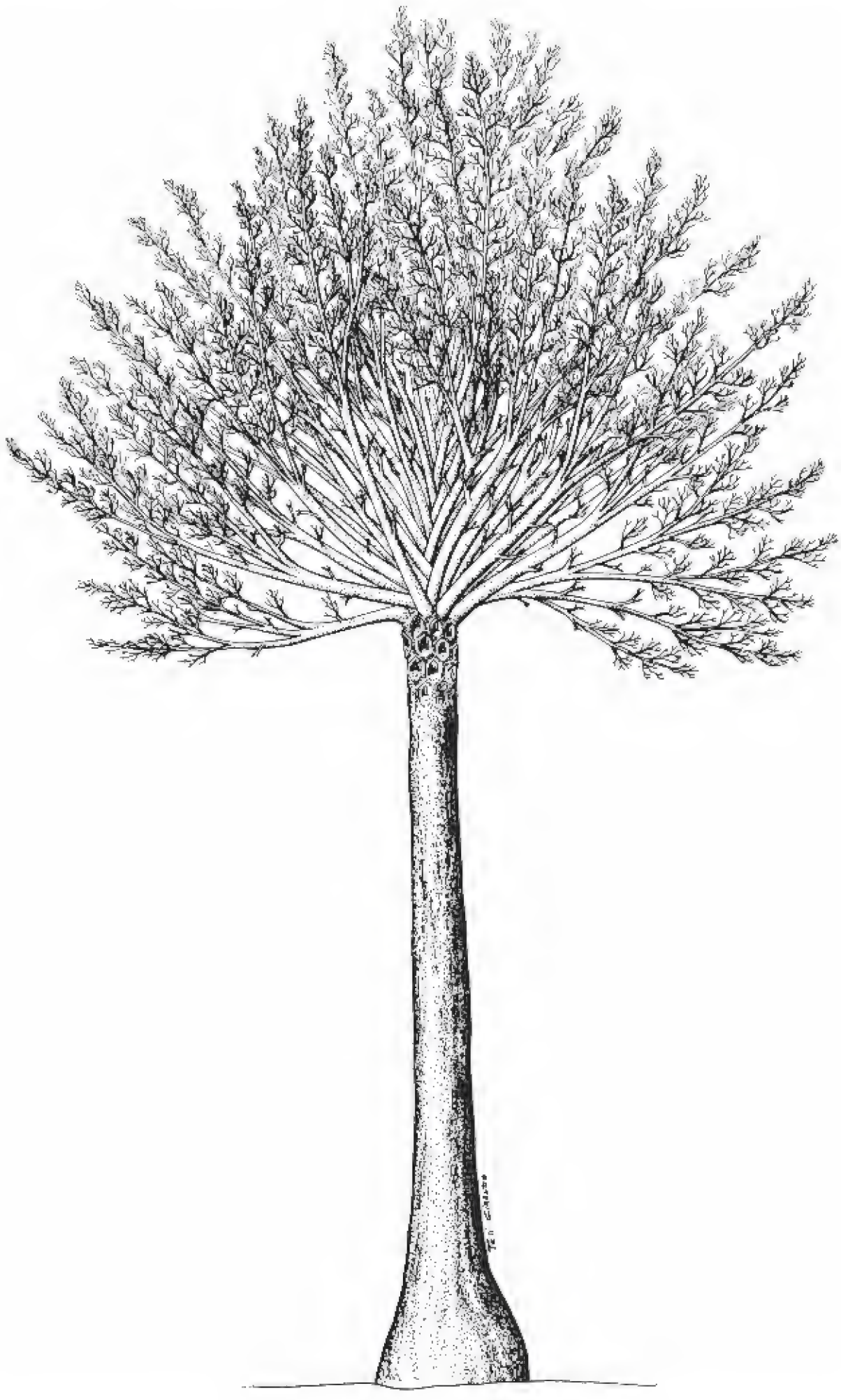

Figure 4. A recent reconstruction of the cladoxylopsid *Pseudosporochnus*, showing its crown of relatively large branch systems, each of which is closely dichotomous so as to appear digitately divided. Reproduced from Berry and Fairon-Demaret, 2002, Figure 9, Int. J. Plant Sci. 163(5) 699-713. © 2002 by The University of Chicago.

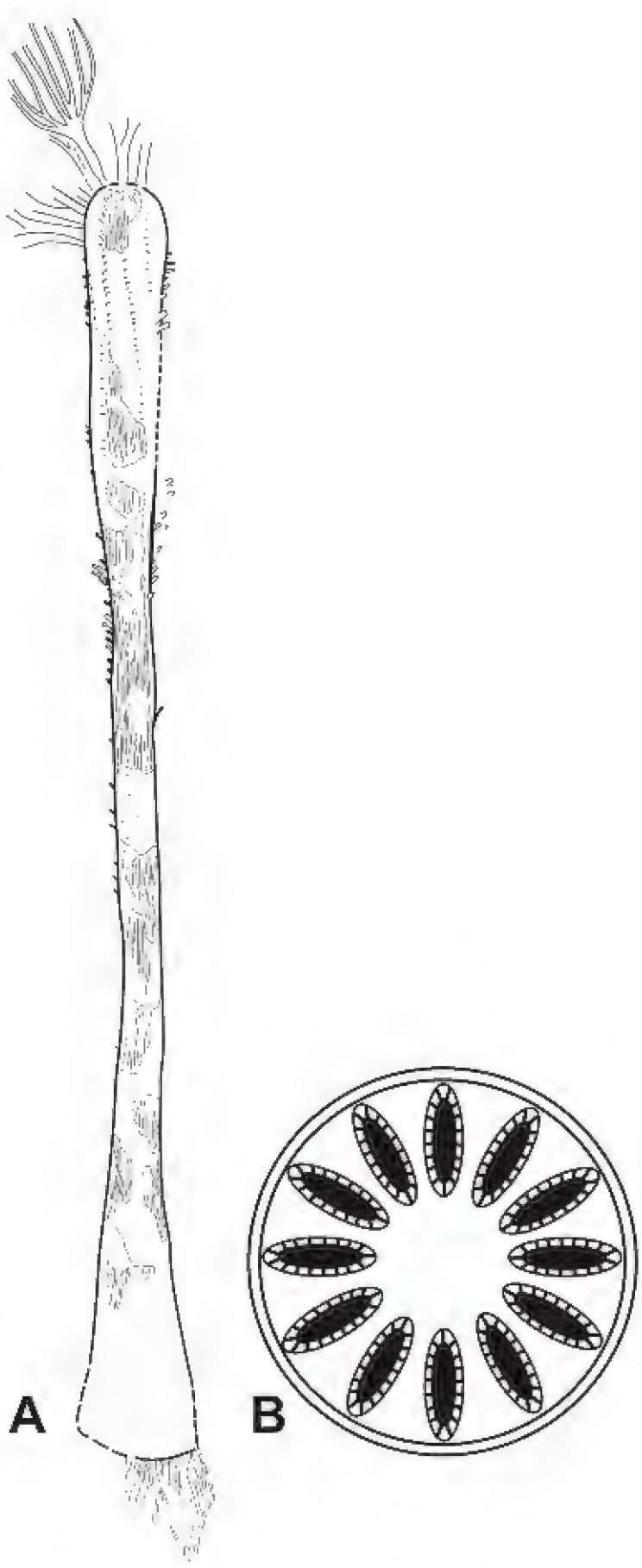

Figure 5. *Calamophyton primaevum*. Drawing of a stem showing two features- the remnant bases of branches along the stem and parts of the attached, digitately arranged branches at the crown. B. Suggested anatomy of *Calamophyton (Duisburgia)* based on Mustafa (1978) showing primary xylem in black, surrounded by aligned, possibly secondary xylem (lines). Redrawn from Giesen and Berry, 2013; stem is from Figure 5a (in part), anatomy redrawn from Figure 17d.

to a large pith in main trunk (Figure 5) as in the presumed base of *Calamophyton* stem (Giesen & Berry, 2013) and *Eospermatopteris/Wattieza* (Boyer & Matten, 1996). Opinion differs as to whether some of these plants exhibit a limited amount of aligned metaxylem or secondary tissues. Coeval progymnosperms possess a eustele and bifacial vascular cambium. Habit, branching, aspects of rooting and internal construction of the large pseudosporochnalean cladoxylopsids might mean they already had diverged to the extent that they have little relationship with extant polypodiid or equisetid lineages, but instead represent a distinct lineage that flourished globally, then went extinct without any survivors (see Rothwell & Stewart, 1993).

Iridopteridaleans

Numerous Middle Devonian plants from Laurussia, Gondwana, and China comprise another lineage of Middle to Upper Devonian euphyllophytes, the Iridopteridales (Stein, 1982; Fu et al., 2011) based on anatomy, morphology, or a combination of these preservation forms (Figure 7). These plants as presently known are smaller than cladoxylopsids, consisting of stems with a deeply ribbed mesarch actinostele comprised of two to three centrally united arms, each arm dividing one or more times to form six to twenty arms/ribs depending on the taxon. A single protoxylem region exists near the end of each arm (Figure 7 A-C). Branching pattern is essentially whorled, each protoxylem pole emitting a trace that supplies either a lateral branch or a smaller dichotomous appendage, the two types occurring in the same whorl (Figure 7D). Sometimes branching occurs in only part of a whorl, with successive partial whorls offset (Stein, 1982). Sporangia terminate ultimate dichotomizing appendages borne from second order branches- some upright and some recurved (Berry &Stein, 2000, *Compsocradus*). Other, often less extensively preserved, taxa attributed to the Cladoxylopsida are not treated here. Relationship to Polypodiidae is unclear; depending

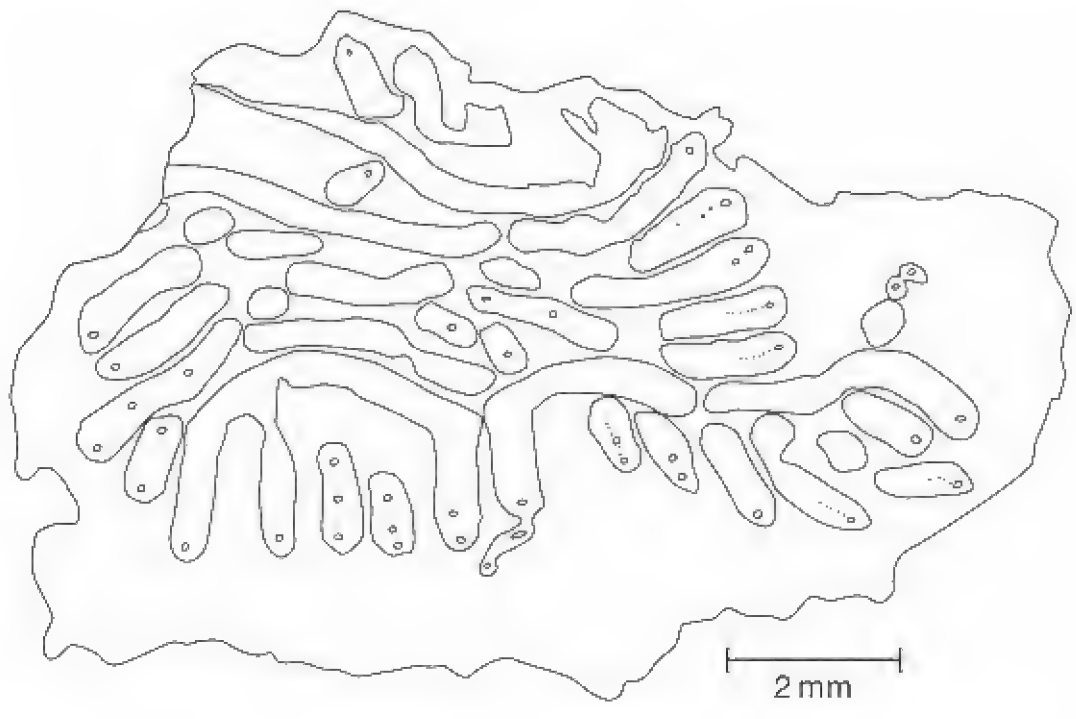

Figure 6. Anatomy of a lateral branch system of *Pseudosporochnus*, which is characteristic of many cladoxylopsids; the vascular bundles are arrayed in a manner interpreted as representing the beginning of a dichotomy. Redrawn from Stein and Hueber, 1989.

on what combination of characters are selected, they may be closer to Equisetidae; if others, of uncertain affinity (Stein et al., 1984; Berry & Stein, 2000).

Rhacophyton and putative relatives

Rhacophyton (several spp) exhibits some characters that presage a fern type of organisation, but also possesses secondary tissues and some morphology reminiscent of progymnosperms or iridopteridaleans. Somewhat rhizomatous to upright axes (?stems) bearing adventitious roots in *R. ceratangium* (Cornet et al., 1976) produce single or paired much divided lateral branch systems which overall appear three dimensional, with more two-dimensional second order axes. The secondary axes depart oppositely, and range from dichotomous to slightly pseudomonopodial. Fertile lateral branch systems are more complex, each pair with one vegetative and one fertile unit. The vegetative segments are elongate and bear divided higher order non-laminate ultimates. The fertile units are much divided and recurved, forming rounded masses of dividing axes terminating in fusiform sporangia with elongate tips. Anatomy of less extensively preserved, more upright appearing stems of *R. zygopteroides* was described by Leclercq (1951) from poorly preserved specimens, and interpreted as an actinostele with unequally sized arms, although these sections have deterioriated so this cannot be confirmed. Similarly arranged lateral branches produce clepsydroid shaped steles. Both were described as surrounded by secondary xylem. In *R. ceratangium*, both main axis and lateral branches are considered to have produced an elongated clepsydroid-shaped primary xylem, surrounded by secondary xylem with rays (Cornet et al., 1976; Dittrich et al., 1983). Traces to higher order axes also are known. *R. condrusorum* Schultka (1978) is similar. *Ellesmeris* Hill et al. (1997) exhibits a simpler, but similar overall architecture, although the ultimate units are laminate, and fertile structures unknown. Anatomy is not intact, but the authors suggest main axis and lateral branch produce an elongate haplostele, with protoxylems near the tips of the arms. Knowing if all orders of branching possessed the same type of stele, or alternatively that stelar configuration changed from radial to

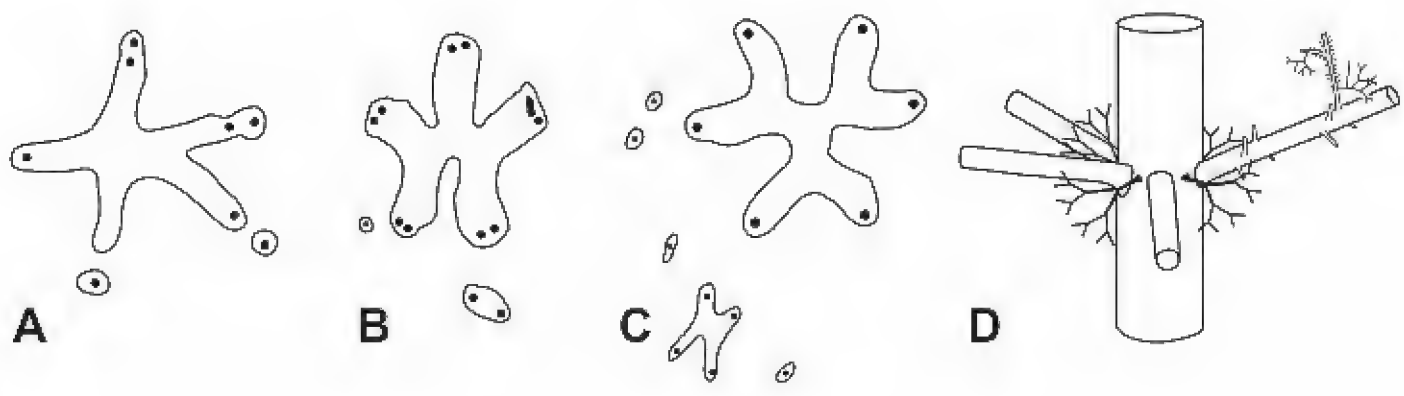

Figure 7. Line drawings of anatomy and stem portion of taxa included in Iridopteridalean cladoxylopsids. A. *Ibyka amphikoma*; B. *Iridopteris eriensis*; C. *Arachnoxylon kopfii*; D. Portion of stem and branching structure of *Anapaulia moodyi*; whorls of branches alternate with dichotomizing structures similar to ultimate branchlets; each lateral branch bears second and ultimate order branches. A-C redrawn from Stein, 1982. D redrawn from Berry and Edwards, 1996.

bilateral from main axis to lateral branch is of some significance in considering relationships and what to interpret as stem vs. leaf in these plants. For the present they usually are considered a separate lineage, Rhacophytaceae, although some authors previously placed them as a basal member of the zygopteridaleans and others consider them more allied to progymnosperms. Berry and Wang (2006) suggest, intriguingly, that *Rhacophyton* shares some characteristics of iridopteridaleans.

STEM EUPHYLLOPHYTES AND RELEVANCE TO EVOLUTION OF LATER FORMS

Trimerophyte grade euphyllophytes exhibit either dichotomous, anisotomous or strongly pseudomonopodial branching of varying degrees of predictable cladotaxy. Some of these terminate in fusiform sporangia, ranging from loosely (*Psilophyton*-grade plants) to densely (*Pertica*) clustered. Anatomy is known from parts of aerial systems of some *Psilophyton* species, while anatomy is unknown in published *Pertica* species. However, *Gothanophyton* and some undescribed plants at the *Pertica*-grade provide data about possible anatomical variation in these entities, showing some differentiation between ribbed main axes and lateral branches, and possibly indicating evolutionary trajectories different from *Pertica*, although affinities of most of these are poorly understood.

Psilophyton-grade basal euphyllophytes

Psilophyton Dawson (Banks et al.) Doran (1980), initially based on dichotomous to slightly pseudomonopodially branched axes, where anatomically preserved, exhibits a haplostele with centrally located protoxylem (centrarch) with little or no differentiation between main axis and lateral branch traces (Figure 8A). It thus appears the least specialized of stem euphyllophytes, but that may be deceptive. At the same time, plants of this type may be more closely related to polypodiids, or lineages leading to polypodiids and equisetids, than many later-appearing taxa.

Over 14 species of *Psilophyton* are now recognized, some based on morphology, one from anatomy only (*P. coniculum*) and others with both, although anatomy so far is not extensively known in most species. Based on current knowledge, preservation ranges from nearly complete plants (*P. crenulatum*) to more fragmentary remains. Phylogenetic analyses involving more than one species of *Psilophyton* indicate the genus is not monophyletic (Kenrick & Crane, 1997; Hao & Xue, 2013) and comparative evaluation of known species further suggests the possibility that these species might represent more than one taxon.

Psilophyton crenulatum Doran (1980), an apparently rhizomatous plant with upright branch systems, exhibits dense regions of mostly vegetative branching, some of which appear immature (apices present) while more distally, spacing of vegetative and fertile lateral branches varies. *Psilophyton dawsonii*, based mainly on anatomy of a 2.5 cm long specimen (Banks et al., 1975), possesses regions of closely spaced vegetative lateral branches. The more distally located fertile lateral branches exhibit a trace suggested to differ in shape from vegetative ones (Banks et al., 1975). Some short dichotomously divided lateral appendages are considered possible aphlebiae or roots. These features are not known for any other *Psilophyton* species, including *P. coniculum* (Trant & Gensel 1985), known only from anatomy (no sporangia attached). In this species, more closely spaced branching occurs mainly in lateral branches.

Comparison of the many other *Psilophyton* species indicates that relative distribution of vegetative vs fertile regions is unknown, partly because the genus is best recognized

morphologically from fertile specimens. In many instances the number of specimens available or mode of preservation limits information. Where numerous specimens are available, study shows branching is variously spaced. Frequently, lateral branches are incomplete and finding completely vegetative branch systems is difficult.

It was previously questioned whether certain previously described species of *Psilophyton* conform sufficiently to the concept of the genus. *P. kräuselii*, from the Middle Devonian of Bohemia (Obrhel, 1959) is too poorly preserved to determine affinity, being based on fragmentary fertile and sterile remains (Hao & Xue, 2013; this paper). Hao and Xue (2008) also question the generic identity of *P. szaferi*, *P. striatum* (Wang & Berry, 2001) and *P. dapsile* (Kasper et al. 1974) because they are either too poorly known or lack twisted sporangia. Examination of *P. dapsile* sporangia in the author's collection suggest that while small, they do show some torsion. *Psilophyton szaferi*, described by Zdebska (1986) from the Lower Devonian of Poland, consists of a portion of a lateral branch system with upright sporangia borne in loose clusters more similar to those in *Pertica* and should be restudied. *Psilophyton microspinosum* from the Early Devonian of Maine (Kasper et al., 1974) exhibits a more rigid pattern of lateral branches and erect, untwisted sporangia, suggesting its affinity should be re-evaluated.

The few morphologically preserved Pragian *Armoricaphyton* and the Emsian unnamed plant from Canada (Gerrienne et al., 2011; Strullu-Derrien et al., 2014; Gerrienne & Gensel, 2015) with an early type of secondary tissue are similar in stem

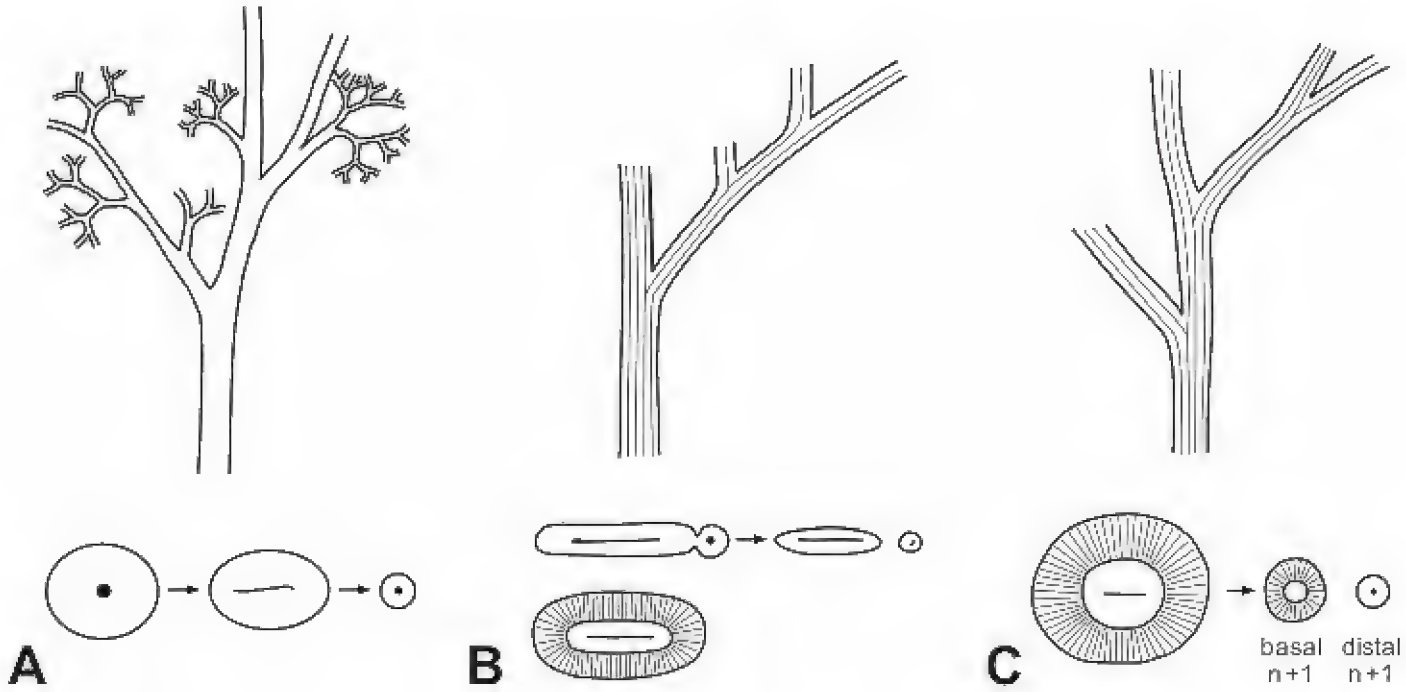

Figure 8. Diagrammatic drawings of small basal euphyllophytes; predominant mode of branching shown above, anatomy below. Axes approx. 2-5 mm. A. a generalized drawing of branch system of *Psilopyton*. Anatomy a centrarch haplostele, producing circular (or possibly square, if fertile) lateral branches. B. Unnamed plant from Emsian, New Brunswick (Gensel in prep). Ribbed, twice branched axes known. Some stems with elongate haplostele producing lateral trace, others indicate same type of haplostele surrounded by secondary xylem. C. *Armoricaphyton chateaupannense*, Pragian, France (Gerrienne et al., 2011; Gerrienne and Gensel, 2016). Ribbed, twice branched axes. Primary xylem a round haplostele, surrounded by secondary xylem. Basal part of lateral branch with secondary xylem, more distal region without. Dark circles or lines in center of steles indicate protoxylem, white area primary xylem, lines = secondary xylem.

diameter and branching, to *Psilophyton* (Figure 8 B, C). No fertile remains are known. All of these taxa, along with *Pertica* type trimerophytes showing preserved anatomy, exhibit P-type tracheid wall patterning *sensu* Kenrick and Crane, 1997 (Gensel, unpublished data). This character may be less useful at lower taxonomic levels, as the combination of characters evident in these plants suggest broader disparity among these early simpler plants than previously realised.

Morphologically preserved Middle Devonian plants such as *Planatophyton* (Gerrienne et al., 2014), *Pauthecophyton* (Xue et al., 2012), and *Tsaia* (Wang & Berry, 2001) are similar to *Psilophyton* in size and some aspects of branching but differ either in specifics of branching, sporangial orientation and/or dehiscence mode. These may represent distal regions of much larger plants or, alternatively, fragments of plants similar to *Psilophyton* in size. Additional study of these plants may reveal features useful in determining if they represent one, or several, Lower to Middle Devonian genera and/or lineages and clarify their postulated phylogenetic relationships.

Larger basal euphyllophytes similar to *Pertica*

Pertica and similar-appearing plants (Figure 9A-C) are taller and more robust in width, with a dominant main axis and variously arranged lateral branches. *Pertica* spp. exhibit mostly isotomously divided lateral branches with terminally borne, closely spaced, erect fusiform sporangia. Anatomy is unknown in published *Pertica* species.

A new genus, initially considered *Pertica*-like but definitely an unrelated taxon (Figure 9A), has a dominant main axis, helically arranged lateral branches that are single or paired, and, in contrast to *Pertica*, dichotomously- divided ultimate units located along their length, some sterile and some terminating in fusiform sporangia. Main axis anatomy is an actinostele (usually) with a single central protoxylem and a protoxylem near the end of each arm (Gensel, 1984). The lateral branch trace(s) are ovoid. There is no evidence of secondary xylem.

An undescribed plant from the Lower Devonian of New Brunswick is similar to *Pertica spp*. in morphology (Figure 9B), with a dominant main axis, helical lateral branches, and dichotomous laterals terminating in tight clusters of fusiform sporangia (Gensel, in prep.). Anatomy is unusual, consisting of a "winged" stele in which four major arms emanate from an elongate central region, but with one arm dividing further (Figure 9B). At least two types of lateral branch traces are formed, differing from main axis stelar configuration, but preservation is incomplete. Do other perticas have similar anatomy or might there be variation?

Gothanophyton Remy et Hass (Figure 9 C), with a 1cm wide main axis and single or paired lateral branches, exhibits a "winged" stele, consisting of an elongated central region with two major arms extending from either end (Remy & Hass, 1986a). One or more of the arms divide again, so that 4-7 armed steles are known. Protoxylems are located at either end of the central region near departure of major arms and near the end of each arm (Figure 9C). Shape and/or lobing of lateral branch traces differ from the main axis configuration. Morphology is poorly preserved, but the authors suggest lateral branches departed singly, in pairs or in threes. Much more needs to be learned about this complex Lower Devonian plant.

In all of these plant types, protoxylems emanate from one or more of the main axis arms to supply lateral branches and only primary xylem is present. Architecture of these plants is morphologically and anatomically plastic, although with some common patterns, but evidence is building that these taxa exhibit enough differences to not be closely

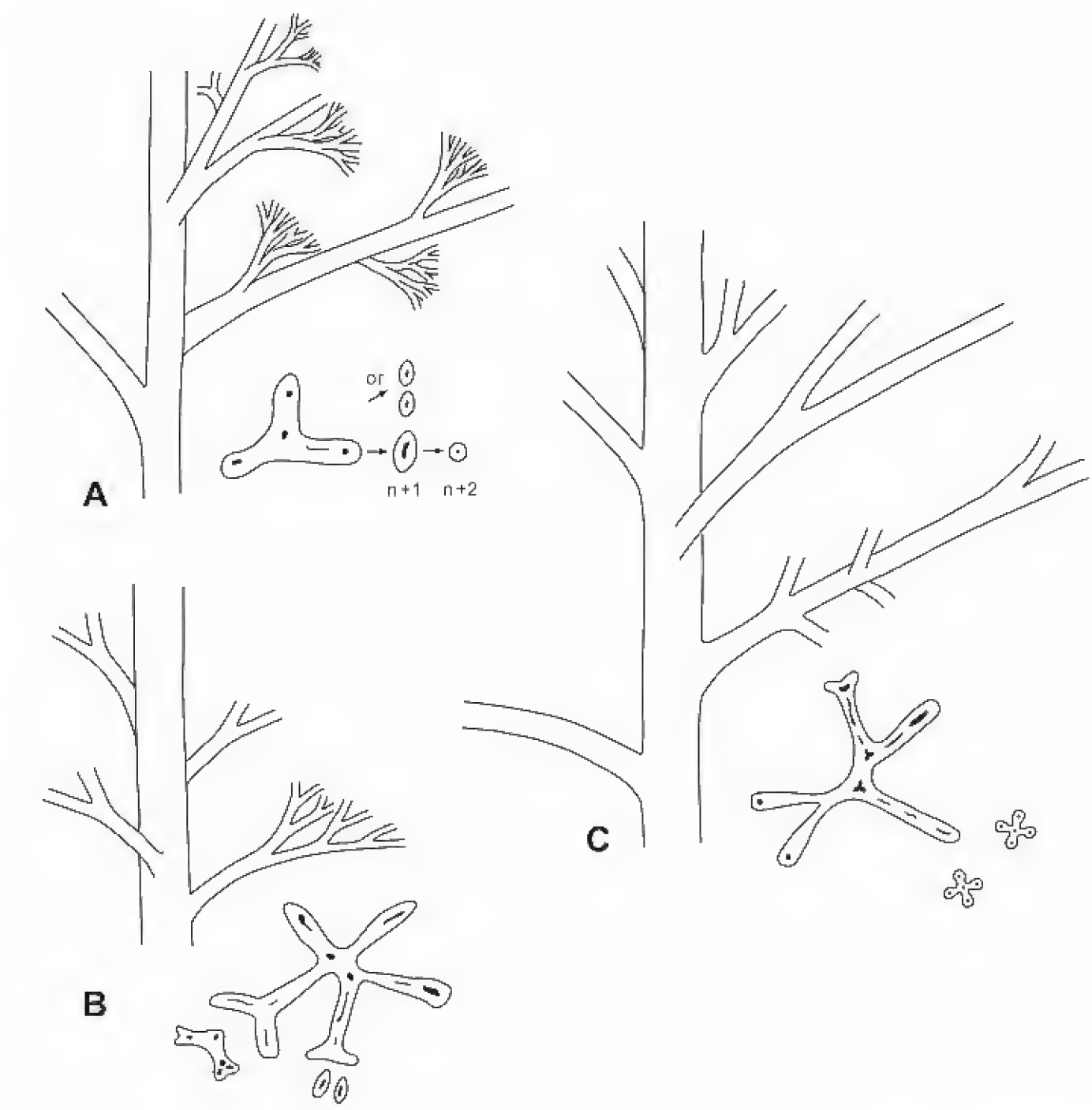

Figure 9. Diagrammatic drawings of larger basal euphyllophytes, similar in size to *Pertica* (main axes approx. 1 cm) - note that none show evidence of secondary xylem. A. Plant described by Gensel (1984), with large main axis, single or paired lateral branches, dichotomously divided ultimates arranged along lateral branches. Anatomy a three-lobed stele with central protoxylem and protoxylem at end of each arm. First order lateral trace (or pair of traces) oval, second order trace circular. (Gensel, in prep) B. A *Pertica*-like plant with large main axis, helically arranged predominantly dichotomously divided lateral branch, terminating in bunched sporangia (not shown). Anatomy consists of slightly elongate central region from which four arms emanate, one of these divides again to produce five arms total. Protoxylem located at each end of central area, and ends of arms. First order traces are elongate to v-shaped or oval and in pairs as shown. Gensel (in prep). C. *Gothanophyton zimmermanni* Remy and Hass. Large main axis with single or paired lateral branches (poorly preserved), bearing at least second order ones. Main axis stele consists of an elongate central region from which 4-7 arms emanate. Protoxylem located at each end of central region, along and at ends of arms. Lateral traces change shape after departure to become 4-lobed. Protoxylem in black. Clear area in stele is primary xylem. C. redrawn from Remy and Hass, 1986.

related. They are similar in showing a change in shape between main axis and first-order lateral branches which conforms more closely to that seen in many ferns or extinct fern-like plants than anything known in progymnosperms, despite apparently comparing more closely to the radiate protoxylem group.

Basal regions or rooting structures for *Psilophyton* and the taller euphyllophytes mentioned above are unknown. The fossils appear to represent aerial shoots extending from a rhizome, but it also is possible that some represent the main part of a non-rhizomatous plant that may have had a basal rooting system. Fairly wide, branched but detached rooting structures running perpendicular to bedding surfaces occur in sediments near some of the larger taxa in northern New Brunswick, while one long, downward extending axis was discovered by Elick (1998) in Gaspé which may represent a rooting structure.

Basal euphyllophytes *incertae sedis*

Several upper Lower Devonian plants (late Emsian) have been excluded from phylogenetic studies, largely because their anatomy is unknown and branching pattern and other features are unique. These include *Oocampsa catheta*, interpreted as a trimerophyte-progymnosperm intermediate (Andrews et al., 1975), *Chaleuria cirrosa* (Andrews et al., 1974) and an undescribed plant with similar branching architecture from northern Maine (Gensel, in prep). They all exhibit a robust, fairly long, greater than 1 cm wide dominant main axis with densely spiraled lateral branches. In *Oocampsa*, higher order branches are several times irregularly dichotomous, bearing clusters of ovoid sporangia. *Chaleuria* and the new plant from Maine exhibit isotomous second-order branches, some terminating in fusiform to ovoid sporangia. Basal regions and thus growth habit(s) are unknown. One might expect either a multilobed or cladoxyl- like stelar configuration based on the major branching patterns. *Oocampsa* differs from the other two in higher order branching and sporangial morphology, while the other two represent one or more as yet unrecognized lineages, or may be early members of one of the later-occurring lineages such as cladoxyls or iridopterids.

Stenokolealeans are still poorly known, but recent studies indicate a possible affinity to early seed plants (Moment et al., 2016).

SUMMARY

Significant new data now exist concerning the time of appearance of plants and morphology and anatomy of some taxa. Exceptionally preserved plant remains have indicated an earlier advent of plants, plant structures and habit, including transitions to upright, even arboreal types by the Middle Devonian. Limited evidence indicates a wider array of growth strategies among these. Although the euphyllophyte and lycophyte lineages separate very early in time, with each demonstrating acquisition of major plant structures, gaps in preservation preclude establishing evolutionary trends or clear phylogenetic relationships within each lineage, especially early vs later Devonian plant types, and between Devonian and Carboniferous or younger lineages that appear more directly related to extant ferns or lycophytes (similar situation for seed plants). As whole plant reconstructions are made and new approaches employed, with new discoveries or re-investigation of earlier material, assessment of character homology will be more possible.

Several putative candidate lineages leading to equisetids or polypodiids remain, but it is clear that some, such as cladoxyls, are already highly specialized in development

and construction by the Middle Devonian. Were more basal taxa similar, as suggested by *Lorophyton* Fairon-Demaret et Li (1993), *Foozia minuta* Gerrienne (1992) or perhaps as yet unrecognized taxa? Basal euphyllophytes at the *Psilophyton, Pertica*, and *Gothanophyton* grade are increasingly interesting in that it is among plants of this type that early differentiation of anatomical configurations of main axis- lateral branch is established. Within this group one might possibly be able to discern early divergence of the fern *sensu lato* vs seed plant lineages. Further examination of anatomy and organisation of lateral branch architecture, incorporating signals of developmental differences, may aid in recognizing early stages in evolution of leaves- these clearly will be different in various lineages. Similarly, the questions of transitions between predominantly rhizomatous and upright habit, and between types of rooting and of organisation of reproductive structures in both major lineages may become clearer with future discoveries or new approaches.

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A SHORT BIOGRAPHY OF THE AUTHOR

Patricia G. Gensel is a professor in the Biology Department, University of North Carolina at Chapel HiIl, having started there in 1975. She received her B.A. at Hope College, Holland, Michigan, then she worked as a research assistant in the Geology Department, King's College, Univ. of London, England, for Dr. John Richardson in the area of Devonian palynology. Patricia returned to the U.S.A. and embarked on graduate studies of fossil plants and their spores with Professor Henry Andrews at the University of Connecticut, Storrs, CT., where she earned a M.S. and then Ph.D. After a few years of post-doctoral work, again with H. N. Andrews, she joined the faculty of (initially) the Botany Department, and later, Biology Department, at UNC.

Patricia continued work on both Devonian (from U.S. and Canada) and Early Carboniferous plants (from US), studying both megafossils and where possible, in situ spores. She also had students who worked on Cretaceous fossils from North Carolina and prepared a field guide to Triassic Plants of the Deep River Basin, NC. She co-authored the book *Plant Life in the Devonian* with H.N. Andrews (1984), and co-edited a book of contributed chapters entitled *Plants Invade the Land* with Dianne Edwards (2001).

She has authored or co-authored at least 68 peer-reviewed publications, the majority on Devonian plants, but also some about in situ spores, spore ultrastructure, and Early Carboniferous plants, several book chapters, reviews or field guides. Patricia has organized many symposia, mostly about early land plant evolution, trained several MS students and a few PhD students. Her major interests center on producing a better understanding of the morphology, structure, evolutionary relationships or patterns of evolutionary change, and where possible ecology, of early land plants, mainly of Devonian and Early Carboniferous age. She continues her interest in palynology, especially in situ spores or spore ultrastructure, in terms of what they may contribute relative to systematics or phylogenies of early plants. Recent publications include a report of early wood from the very Early to Early Devonian of France and New Brunswick (Gerrienne et al., 2011, Gerrienne & Gensel, 2016, work in prep), a revision of sporangial morphology and attachment in the iconic zosterophyll *Sawdonia ornata* (Gensel & Berry, 2016) and paleoenvironmental interpretations of some of the major plant-bearing deposits in New Brunswick or early coal (Kennedy et al., 2012; Kennedy et al., 2013). Her on-going work focuses on elucidating anatomy and morphology of basal euphyllophytes (formerly trimerophytes), additional work on *Sawdonia* spp., and other Devonian or Carboniferous plants.

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A NEW RECORD OF BROWN ROT DISEASE IN WATER FERN *AZOLLA MICROPHYLLA* (AZOLLACEAE): LOSS OF IMPORTANT BIO-RESOURCE

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Key words: cottony white mycelium; yellow pigmentation; macroconidia; microconidia; chlamydospores; pathogenic fungus; *Fusarium thapsinum*

ABSTRACT

Severely infected water fern, *Azolla microphylla* Kaulf., has been observed in natural habitats in different districts in the lower Gangetic plain of West Bengal, India. The infected plants have turned dark brown and been rotted entirely within 5-7 days, causing loss of natural resource as the plant species has immense commercial value. Specific growing colonies with cottony white mycelium, yellow pigmentation on the cultured medium, macro- and micro- conidial features, and absence of chlamydospore indicate the causal pathogen to be *Fusarium thapsinum* Klittich., Lesile, Nelson, Marases. The identification of the causal pathogen has been authenticated by standard pathogenicity test (Koch's Postulates). Extensive literature survey reveals that this is a new record of brown rot disease in *A. microphylla*.

INTRODUCTION

Azolla Lam. is a free-floating, fast growing water fern. The plant is widely distributed in tropical, subtropical, and warm temperate zones in different countries of the World including India (Wagner, 1997). Here *Azolla* spp. are naturally available, mostly on moist soils, freshwater ditches, ponds, lakes, sluggish rivers and marshy lands. *Azolla* is treated as a 'green gold mine', because of its high nutritive value and it is considered as 'super plant' due to its fast-growing capacity (Wagner, 1997). They can even grow in nitrogen deficit areas and can double the biomass in 3-5 days because of its unique symbiotic relationship with the nitrogen fixing endophytic blue-green cyanobacterium, *Anabaena azollae* Sterberger (Wagner, 1997).

High nutritive values (Buckingham et al., 1978; Alcantara & Querubin, 1985; Paoletti et al., 1987; Lejeune et al., 2000; Alalade & Lyayi, 2006) and huge productivity through easy cultivation methods (Dao & Tran, 1979; Lumpkin & Plucknett, 1982; Liu et al., 2008) are the key factors for utilizing *Azolla* as one of the most promising aquatic plants for livestock feed (Becerra et al., 1995; Alalade & Lyayi, 2006; Cagauan & Pullin, 1991; Shiomi & Kitoh, 2001; Fiogbe et al., 2004), and as an effective natural fertilizer to increase rice yield successfully (Peters, 1978; Tung & Shen, 1985; Watanabe & Liu, 1992; Yadav et al., 2014). The plant has been employed in experiments during space and planetary travel (Liu et al., 2008; Carrapico, 2002; Katayama et al., 2008), and used for the production of non-polluting high energy biofuel (Peters et al., 1976; Newton, 1976; Das et al., 1994; Hall et al., 1995), especially biodiesel (Salehzadeh et al., 2014). The plant can act as reducer of various greenhouse gases by ammonia volatilization

(Watanabe & Liu, 1992), by inhibiting methane production from rice field (Prasanna et al., 2002), and noticeably by sequestering atmospheric CO_2 (Brinkhuis et al., 2006). In addition, weed control (Krock et al., 1991), mosquito repellence (Ansari & Sharma ,1991), phyto-remediation from waste water (Jain et al., 1989; Saxena, 1995; Costa et al., 1999; Antunes et al., 2001; Khosravi et al., 2005; Umali et al., 2006; Rai, 2008; Costa et al., 2009; Rai & Tripathi, 2009; Elmachliy et al., 2010; Rai, 2010a, b; Sood et al., 2012) and soil (Umali et al., 2006; Cohen et al., 2002 and Mashkani & Ghazvini, 2009) are added approaches for utilizing this vital bio-resource.

With view to the many valuable uses of *Azolla*, the present authors have attempted to explore the species under various abiotic stresses through experimental set-up by culture of collected specimens from natural habitats of the lower Gangetic plains of West Bengal, India. During field survey, it has been observed that among the available *Azolla* species, *A. microphylla* Kaulf. grows dominantly over others and it can resist direct sunlight in summer. However, the species has been found to be severely infected by a fungal pathogen at the end of winter season (January-March) in most of the collection sites, which disrupts normal growth of the plant and ultimately causes death within 5-7 days.

From the available literature, it seems that pathogenic reports on *Azolla* spp. are rather meagre (Sasi et al., 1982; Kannaiyan, 1985; Kannaiyan & Kumar, 2005; Lee et al., 2011; Banihashemi, 2014). The present communication describes a new causal organism for *A. microphylla*, which may help in identifying suitable measure(s) to protect this important natural resource.

MATERIAL AND METHODS

Fresh and infected samples of *Azolla microphylla* Kaulf. were collected from different sites of three districts, namely, North 24 Parganas (22.936 N, 88.392 E; 22.825 N, 88.786 E), South 24 Parganas (22.021 N, 88.615 E), and Nadia (22.983 N, 88.445 E) in the lower Gangetic plain of West Bengal.

The morphological studies of infected and non-infected plants were made by hand lens and stereo zoom microscope (model LEICA S8APO). The anatomical details of infected plants and that of the isolated pathogen were thoroughly studied under light microscope (model Leitz Laborlux S). Macro and micro photographic documentations were made using Nikon D3200 DSLR and EC3 scientific cameras. To identify the pathogen from morphological characteristics, first the isolations were made from the frond of the naturally infected *A. microphylla* plant using a sterile inoculating loop and plated onto 2.25% Potato Dextrose Agar (PDA). A total of five replicas of culture and three replicas of control plates were incubated at 25˚C temperature in complete darkness. After 2–3 days of inoculation, the fungal colony was observed to grow in each of the five replications of cultured plates but no changes were observed in control plates. The colony morphology, growth pattern, size and shape of macro- and micro- conidia were characterized thoroughly.

To confirm the identity of the pathogen, the standard pathogenicity test (Koch's Postulates) was performed stepwise. Student's *t*-test was done to compare the variations in two sets of data of pathogens detected from inoculated and re-inoculated pure cultured plates prepared through the pathogenicity test. The tests were performed by inoculating naturally-collected, healthy *A. microphylla* plants of a single genotype, because the disease was observed in *A. microphylla* only in natural habitats. A total of 45 experimental plants were surface sterilized through rinsing in 70% ethanol for 30 sec. followed by 1%

sodium hypochlorite solution for 1 min., and sterile distilled water for three times. Out of 45 surface sterilized plants, 30 healthy plants were wounded on the fronds by sterile needle. A small mycelial plug taken from a month-old colony of the pathogen was inserted into the wounds using a sterile toothpick. Both 30 inoculated and 15 non-inoculated plants were then placed onto nutrient medium, distributing five plants in each plate so that six replicas were made for inoculated plants and three for non-inoculated ones. The nutrient media was prepared using HIMEDIA PTO10 Murashige and Skoog medium with 17.04 gm./lit. and 10 gm. of agar. The plates were kept in a growth chamber at 25˚±2˚C temperature and 1800 lux intensity of light in 16:8 hr day and night cycle.

RESULTS AND DISCUSSION

Severe infection was observed in *A. microphylla* during the months of January to March in the field (Figure. 1A, B), as well as in a tank placed in the experimental garden of the Botany Department, Kalyani University (Figure 1C). In a fresh individual plant (Figure 1D), at first the infection was observed in roots (Figure 1E), followed by dark brown-coloured spots on the leaf (Figure 1F). Later, the infected areas sequentially enlarged (Figure 1G), and eventually the whole leaf turned into brown, showing bloch notch symptoms (Figure 1H). Finally, the plant rotted within 5-7 days (Figure 1I). Outwardly protruding, cottony, white mycelial growth covering the entire leaf surface was observed with disease severity (Figure 1J). The transverse section of infected leaves at the initial stage showed loosening of leaf tissues with distinct entrance of hyphae (Figure 1K). Anatomical section of leaf was not possible at the final stage of infection due to total disintegration of leaf tissues. The browning and rotting of leaves and roots are characteristic symptoms produced by the pathogen in *A. microphylla*. The dark brown coloration of the infected parts of the plant is because of the degradation of chlorophyllous pigments and probably production of phenolic compounds as resistance to pathogens (Lattanzio et al., 2006). By comparing the symptoms of rot diseases (Agrios, 2005), the studied infection in present investigation has been designated as brown rot disease of *A. microphylla*.

Each replica of cultured PDA media produced fungal colonies with very thick, abundant, cottony white mycelium, finally attaining a diameter of 48.71±3.0 mm (Figure 1L) after seven days of incubation. The growth pattern of the mycelium was radial. A characteristic yellow pigmentation was found to appear on the agar plate (Figure 1L) after 3-4 days of incubation. After 5-6 days, a slight purplish pigmentation was observed to ooze out and persisted for 1-2 days only. No sporodochia was observed in any of the cultured plates.

Under the microscope, the fungal hyphae appeared hyaline and septate (Figure 1M). Conidiogenous cells were mostly monophialidic (Figure 1N) and rarely polyphialidic. Monophilalidic conidiogenous cells were long (range 10.53-33.94 µm), with an average length of 21.43±7.41 µm (Table 1). The microscopic examination of the fungi taken from the pure culture plates revealed hyphae with characteristic macro- and microconidia (Table 1). Microconidia were produced in relatively short chains (Figure 1O) or in a false head; conidiophores were branched or unbranched, bearing hyaline, monophialidic, conidiogenous cells. Three different forms of microconidia [oval (Figure 1P), fusiform (Figure 1Q), and napiform (Figure 1R)] were found, which were hyaline, thin-walled, and yellowish in colour. Fusiform shaped microconidia had a single septum. The average sizes of the oval, fusiform, and napiform shaped microconidia were

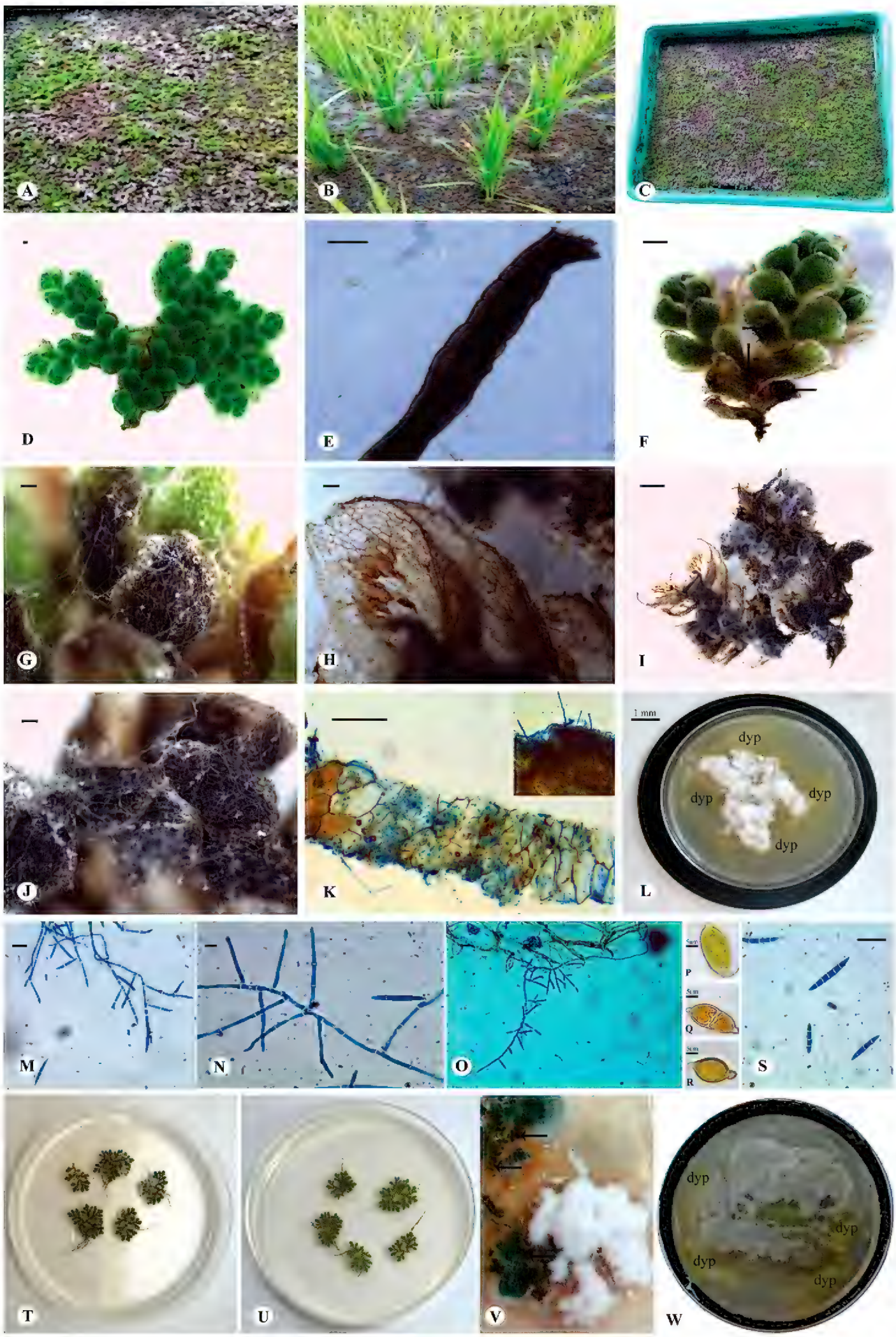

Figure. 1(A.-W.): Caption opposite page

10.73±3.31x2.48±0.33 µm, 13.27±1.98 x 4.85±0.48 µm, and 7.17±0.77 x 3.37±0.18 µm respectively (Table 1).

The macroconidia were found abundantly in aerial parts of the hyphae of the infected leaf; 3-5 septate, but rarely 6; straight to falcate, thin walled, hyaline; slightly curved and tapering in a banana shape (Figure 1S). The average size of macroconidia is of 33.10±5.92 x 3.62±0.28 µm (Table 1). The apical cell of each macroconidia was conical, whereas the basal cell was relatively ill-developed and not the usual foot-shaped. The average length of the apical cell (6.47±0.77 µm) was longer than the penultimate cell (5.29±0.83 µm) (Table 1). It is significant to note that no chlamydospore was found in any one of the culture plates.

The growth pattern of the cultured pathogen onto PDA media and the occurrence of distinctive banana-shaped macroconidia and specific types of microconidia indicate that the causal pathogen is *Fusarium* Link (Moretti, 2009). *Fusarium* is a well-known plant pathogenic fungus, which is soil borne in nature and causes severe plant diseases around the world (Agrios, 2005). The *Fusarium* strain that produces diffusing yellow pigment was separated as a new species, *F. thapsinum* Klittich., Lesile, Nelson, Marases (Teleomorph, *Gibberella thapsina*) (Klittich et al., 1997, Summerell et al., 2003; Leslie & Summerell, 2006).

The characteristic features of cottony white mycelium, yellow pigmentation onto pure culture PDA medium, absence of chlamydospores, shape and size of macro- and microconidia corroborate with the diagnostic features of the holotype BPI 737885 (Klittich et al., 1997) and hence identify the species epithet of the present causal pathogen as *F. thapsinum*.

After seven days incubation in the pathogenicity tests, identical disease symptoms with brown-coloured lesions and protruding cottony white mycelium were observed only on the inoculated plants (Figure 1V) and the fungus was consistently re-isolated from lesions. The identity of the re-isolated pathogen was confirmed as *F. thapsinum* again by pure culturing onto PDA medium (Figure 1W) maintained at similar conditions mentioned before and subsequent microscopic examinations of the hyphae with

Figure 1(A.-W.): Non-infected, and infected *Azolla microphylla* plant showing symptoms of brown rot disease, cultured colony of causal pathogen, and microscopic observation of different features of causal organism (bar= 0.1 mm except otherwise mentioned). A. & B. Naturally infected plant on pond (A) and in rice field (B); C. Infected plant grown in tank at experimental garden of Botany Department, Kalyani University; D. Non-infected plant; E. Infected root showing brown coloration; F. Brown coloured spots on leaf (arrow marked) at initial stage of infection; G. Gradual enlargement of infected area on leaf; H. Leaf showing bloch notch symptom; I. Entirely rotten plant at final stage of infection; J. Cottony white mycelial growth on infected leaf; K. Transverse section of infected leaf showing hyphal infiltration; L. Growth pattern of pure cultured colony showing white mycelium and diffuse yellow pigmentation (dyp); M. Septate fungal hyphae; N. Monophialidic conidiogenous cells; O. Occurrence of microconidia in short chain; P.-R. Microconidia of different shapes- P. oval, Q. fusiform, R. napiform; S. Banana-shaped macroconidia; T. Experimentally diseased plants in pathogenicity test; U. Control plants in pathogenicity test; V. Magnified view of experimentally diseased plants showing same disease symptoms; W. Growth pattern of pure cultured colony prepared from re-isolated pathogens from experimental disease plants showing white mycelium and diffuse yellow pigmentation (dyp).

Table 1: Characterization of macro- and micro- conidia grown in pure cultures prepared from the isolated inoculum of naturally diseased plant (NDP) and the re-isolated inoculum of experimentally diseased plant (EDP) of *Azolla microphylla*

Attributes	Measurement (in µm) of macro- and micro- conidia				*t value at 18 df
	NDP		EDP		
	Range	Mean ± SD	Range	Mean ± SD	
MACROCONIDIA					
No. of septation	3 - 6	5.00± 0.79	3 - 6	5.00± 0.94	---
Length of apical cell	4.82 – 7.58	6.47 ± 0.77	4.78 – 7.83	6.27 ± 1.14	0.4485
Length of penultimate cell	3.89 – 6.51	5.29 ± 0.83	4.12 – 6.62	5.00 ± 0.78	0.7648
Ratio of apical to penultimate cell	0.95 – 1.58	1.25 ± 0.22	0.98 – 1.65	1.26 ± 0.18	0.1004
Size [length (l) x width (w)]	19.43 – 39.23 (l) 3.06 – 3.98 (w)	33.10 ± 5.92 x 3.62 ± 0.28	21.26 – 40.79 (l) 2.80 – 4.00 (w)	32.53 ± 4.66 x 3.61 ± 0.35	0.2319 (l) 0.0341 (w)
Length width ratio	7.63 – 11.50	9.18 ± 1.70	5.84 – 11.39	9.09 ± 1.57	0.1253
MICROCONIDIA					
Length of conidiogenous cell	10.53 – 33.94	21.43 ± 7.41	10.96 – 34.08	22.42 ± 6.87	0.3012
Size of oval shaped microconidia [l x w]	7.22 - 16.08 (l) 2.05 – 2.92 (w)	10.73 ±3.31 x 2.48 ± 0.33	6.99 - 17.09 (l) 1.98 – 3.03 (w)	12.45 ±3.11 x 2.58 ± 0.35	1.1672 (l) 0.6400 (w)
Size of napiform shaped microconidia [l x w]	6.18 – 8.26 (l) 3.14 – 3.68 (w)	7.17 ± 0.77 x 3.37 ± 0.18	6.10 – 8.63 (l) 3.23 – 3.71 (w)	7.47 ± 0.80 x 3.42 ± 0.16	0.8199 (l) 0.5843 (w)
Size of fusiform shaped microconidia [l x w]	10.08 – 16.86 (l) 3.97 – 5.41 (w)	13.27 ± 1.98 x 4.85 ± 0.48	9.77 – 16.23 (l) 4.06 – 5.41 (w)	12.86 ± 2.20 x 4.76 ± 0.49	0.4284 (l) 0.3894 (w)

* $p>0.01$ in all cases

characteristics macro and micro conidia (Table 1). The control plants, however, did not show any disease symptoms and no traces of *F. thapsinum* were recovered from them.

Thorough literature survey reveals that previously reported diseases in *Azolla* are black rots in *A. pinnata* from India (Kannaiyan, 1985), *Rhizoctonia* blight in *A. japonica* from Korea (Lee et al., 2011), and *A. filiculoides* from Iran (Banihashemi, 2014). Kanniyan and Kumar (2005) identified a fungal complex as a causal organism for black rot disease in *A. pinnata*, with *Rhizoctonia solani* as the primary infecting organism.

CONCLUSION

The present investigation reports a new record of brown rot disease in *Azolla microphylla* caused by the fungal pathogen *Fusarium thapsinum*. Because of the immense value of this plant, it will be essential to explore preventive measure(s) to protect this important bio-resource from destructive fungal attack.

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CYSTOPTERIS FRAGILIS SUBSP. *HUTERI*, A TAXON NEW TO THE BRITISH ISLES AND RELATED TAXA IN THE *CYSTOPTERIS FRAGILIS* COMPLEX: *CYSTOPTERIDACEAE* (POLYPODIOPSIDA)

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ABSTRACT

A taxon in the *Cystopteris fragilis* (L.) Bernh. complex that occurs in Britain appears up to now to have been overlooked there. It is proposed that this fern, which was recently rediscovered in Snowdonia, North Wales, should be referred to a subspecies of *C. fragilis*, namely subspecies *huteri* (Hausm. ex Milde) C. Prada & Salvo, that occurs in mainland Europe, but had not previously been recognised in the British Isles. The Snowdonia fern is distinct in its morphology from other British taxa in the *C. fragilis* complex and is described here together with its historic records and present distribution, updated from information given in an earlier paper in *Watsonia* (Tennant, 2010), and its relationship to affiliated taxa is discussed.

INTRODUCTION

The *Cystopteris fragilis* complex is perhaps the most difficult one to resolve of all fern groups. In the British Isles it presently includes the species *C. fragilis* (L.) Bernh., *C. alpina* (Lam.) Desv., *C. diaphana* (Bory) Blasdell, and *C. dickieana* R. Sim. It is generally accepted that more than one original diploid parent was probably involved in the ancestry of the present species recognised in the complex. One result of which is the large variation between and within the species, making it especially difficult to recognise the different cytotypes based solely on their gross morphology. Over many decades, a study was made of the species within the complex, and especially their variants, both in the field and in herbarium collections. This led to earlier papers being published on *C. alpina* and *C. dickieana* in Britain (Tennant, 1995; 1996; 2010), but two especially distinctive taxa were noted in Britain, which in their morphology did not readily conform to the appearance or descriptions of any of the known presently extant British taxa within the complex.

The first of these was found in 2008 in Snowdonia, V.C. 49, North Wales, where it was extremely local and scarce. A search in herbarium collections revealed that a morphologically identical taxon had been collected previously there. A paper published in *Watsonia* (Tennant, 2010) gave most of the records of this fern, which are updated and relisted in this paper, together with its historical references, description, illustrations (Figures 1 & 2), and habitat, and evidence for the presently proposed identity of this Snowdonia fern is given.

The second distinctive *Cystopteris* taxon discovered during the course of these studies occurs in northern Scotland, and a similar taxon also in North Wales. These ferns appear to be affiliated to certain variants currently included under *C. alpina* (Lam.) Desv., previously as *"C. regia"* auct., non (L.) Desv. Morphologically these Scottish ferns

closely match certain *Cystopteris* forms found by the author recently in alpine regions of mainland Europe in three different countries (Figures 3 & 4). Living material and details of these Scottish ferns, which were grown from spores, have been donated to the Natural History Museum in London for further study and will be the subject of a later paper.

Figure 1. *Cystopteris fragilis* subsp. *huteri*. Cultivated examples from Snowdonia, V.C.49, North Wales illustrating the degree of variation. (Further illustrations were given previously in Tennant (2010)).

Figure 2. *Cystopteris fragilis* subsp. *huteri.* Snowdonia, V.C. 49, North Wales. Cultivated specimen.

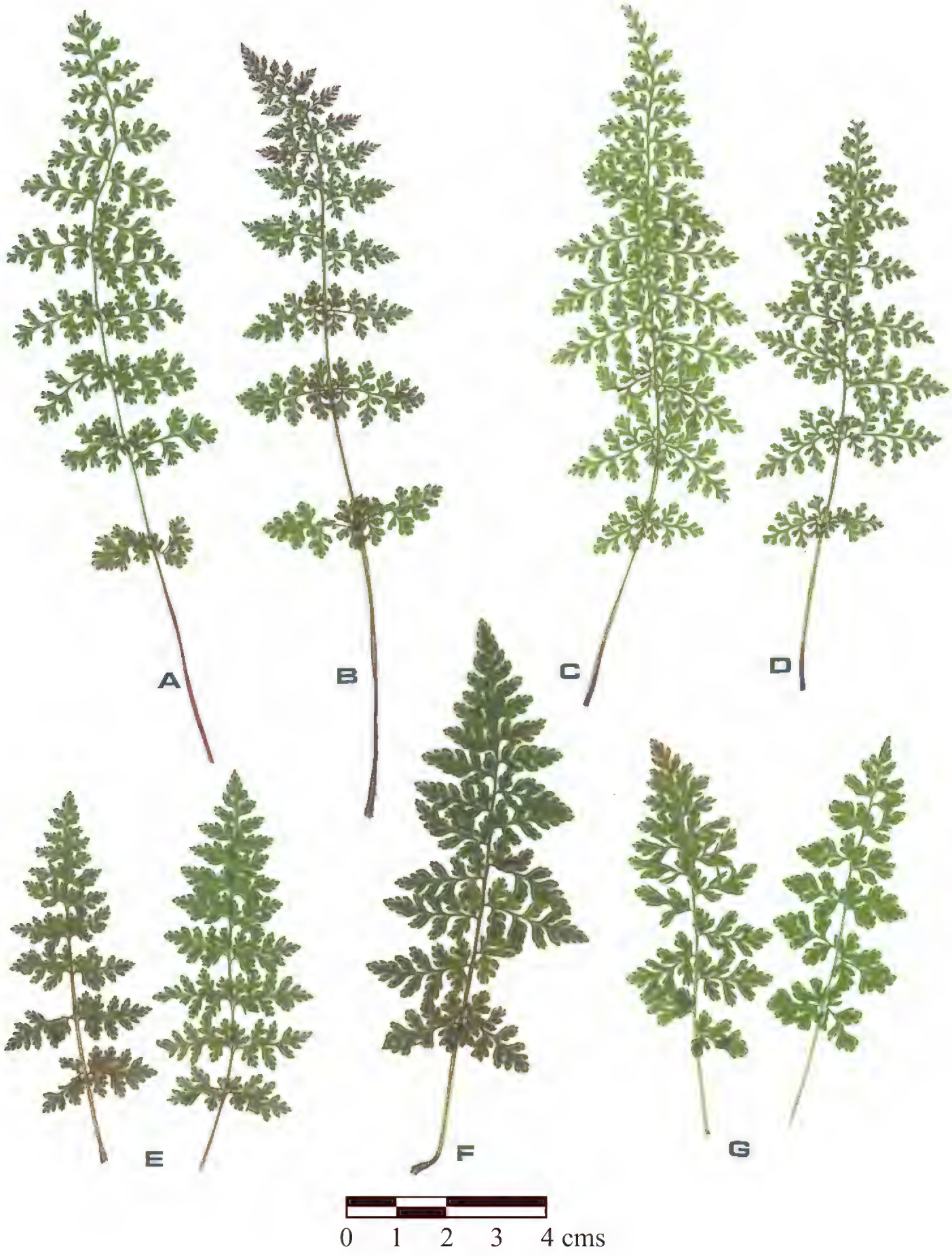

Figure 3. *Cystopteris alpina* (Lam.) Desv. s. str. From the European Alps, A – D. A. France. B. Spain. C. Austria. D. Italy. (British examples from Yorkshire collected by J. Backhouse were illustrated in Tennant (1995)). The *Cystopteris* variant taxon, which is intermediate between *C. alpina* and *C. fragilis*, E – G. E. From the Italian Dolomites. F. French Alps. G. Northern Scotland.

Figure 4. *Cystopteris* variant from Northern Scotland, V.C. 105, which is intermediate between *C. alpina* and *C. fragilis*.

MATERIALS AND METHODS

The fieldwork described below on *Cystopteris* in Britain dates back over many decades through an early interest of the author, particularly in the British records for *C. alpina*. More recently, following several examinations of herbarium specimens at the Natural History Museum (NHM) London (**BM**) and at the Royal Botanic Garden, Edinburgh (**E**) between 2004 and 2006, details of all relevant specimens were abstracted and compared with recently found examples of *Cystopteris.* This was followed by further fieldwork in North Wales and in Scotland. Permission was obtained from the Countryside Council for Wales to collect a small amount of material on Snowdon, V.C. 49, which was undertaken in 2009, following which this Snowdonia fern was cultivated in Yorkshire. Living examples of the Snowdonia *Cystopteris* were then donated to NHM in London for further studies. This work is being undertaken by Dr F.J. Rumsey at NHM, and so far has included a detailed examination of spores and some plastid DNA studies.

Meanwhile, historic publications were examined and information dating back to the 17th century was abstracted, after it was discovered that the Snowdonia *Cystopteris* had first been collected at that time. Field studies on *Cystopteris* were more recently extended in alpine regions of mainland Europe, namely Norway (Tromsø 2009), France (Vanoise Alps 2010), Spain (Central Pyrenees 2012), Italy (Dolomites 2014, 2015), and Austrian Tyrol (2016). Material found there was compared with examples from Britain.

RESULTS

Description of the Snowdonia *Cystopteris*

This fern was largely described and illustrated in an earlier paper (Tennant, 2010) from samples that had been found in Snowdonia in 2008, and cultivated from 2009, although one crucial character was then omitted. During examination of the cultivated material it was noted that the fronds were distinctly glandular, having scattered, capitate glandular hairs on the pinnae, a character unique in British *Cystopteris.* A further more detailed description is as follows, with illustrations given in Figures 1 and 2.

Stipe slender, 1.5 – 6 cm. brownish, with scattered pale brown linear scales, frond (lamina) lanceolate, 4 – 10 x 2 – 5 cm, pinnae mainly narrowly ovate, 1.5 – 2.5 x 0.7 – 1.3 cm, with scattered, capitate glandular hairs, which are most visible on the margins, pinnules stalked, the lower oblong-obdeltoid, subtruncate at the apex, often with short, sharply acute points, the upper narrower, linear-obdeltoid, more acute at the apex, the veins mainly terminating in the acute apical points. Sori scattered, situated adjacent to the veins, spores echinate, very large, mean c. 50 microns (F.J. Rumsey, pers.comm.).

The juvenile sporophytes have well-separated (divaricate), linear-oblong, blunt pinnules, unlike those of typical *C. fragilis*, but very similar to those of *C. alpina.* The immature, intermediate fronds usually have either obdeltoid (wedge-shaped) or linear pinnule lobes, again similar to those seen in *C. alpina*. The mature fronds are more finely dissected, sometimes with pinnae obliquely inclined to the plane of the lamina. The Snowdonia ferns, although sharing several characters with *C. alpina* are readily distinguished from it by the acute points at the apex of the pinnule lobes into which the majority of the veins terminate, and the unique presence of glandular hairs on the pinnae. Plastid DNA examination has also confirmed that the ferns are not *C. alpina* (F.J. Rumsey, pers. comm.). The relationship of these Snowdonia ferns to a described, glandular subspecies of *C. fragilis*, which occurs in southern, central and western montane regions of mainland Europe is discussed below.

Habitat

The Snowdonia fern is a plant of the alpine zone in Britain, probably not occurring at altitudes below 700m. In the two localities in which it was rediscovered in 2008 it was extremely local and scarce. It occurred on basic rocks in a north-facing vertical crevice and in a recess on the cliff face. No other vascular plants were present, but *Cochlearia pyrenaica* subsp. *alpina* (Bab.) Dalby, *Minuartia verna* (L.) Hiern and *Silene acaulis* (L.) Jacq. occurred nearby, and typical *C. fragilis* was not far distant.

Records of the Snowdonia *Cystopteris*

Specimens in British herbarium collections and references in historical literature showed that the fern that had been rediscovered in Snowdonia in 2008 had been collected very much earlier. Identical material was located by the author in 2004-2006 at the Natural History Museum (NHM) in London (**BM**) and other relevant *Cystopteris* specimens were found in the herbarium of the Royal Botanic Garden (RBGE) in Edinburgh (**E**), and the Snowdonia records are given below. This fern was first collected in the late 1680s by Edward Lhwyd in Snowdonia, and two of Lhwyd's original specimens were recently located by F.J. Rumsey in the Sloane herbarium at **BM**; these specimens were identical to those found on Snowdon by the author in 2008. Further specimens collected by Lhwyd, which are probably the same, are present in the herbarium of Oxford University (**OXF**) (Edgington, 2013). Lhwyd's specimens were also sent to John Ray and details were included in Ray's *Synopsis* (Ray, 1724). Lhwyd's record and others from Snowdonia were later included by Sir J.E. Smith (1828; 1834) in his *English Flora,* but under *Cystea regia* Smith, possibly the first, but illegitimate, binomial name given to this fern, which is discussed later in this paper.

The Snowdonia *Cystopteris* would meet the I.U.C.N. Threat Category of 'critically endangered', and is especially vulnerable to collecting, therefore precise details of its locations are withheld, but otherwise its presently known records are as follows:

Snowdonia, SH65, Caernarvonshire, V.C.49 circa 1688, Edward Lhwyd (Sloane herb., **BM; OXF**)

Snowdon, SH65, V.C.49, 1824, collector unknown, herb.Rev. William Newbould as *C. fragilis* (**BM**)

Snowdon, 1826, William Wilson (?herb. J.T.I. Boswell-Syme), as *Cyathea regia* (in the *C. fragilis* folders) (**BM**)

Snowdon, 1847, collector uncertain, herb. F.J. Hanbury, as *C. fragilis* var. (**BM**)

Near Glyder Fawr (Cwm Idwal), SH65, V.C.49, 1853, collector's name illegible, in *C. alpina* folder (**BM**)

Cwm Glas, Snowdon, 1874, collector uncertain (same sheet gives H. & J. Groves also W.R. Linton), as *C. fragilis* (**BM**)

Snowdon, 1890. J. Lloyd Williams, in herb. Arthur Bennett, as *C. alpina* (later renamed *C. fragilis*) (**BM**)

Snowdon, 2008, 2009, D.J. Tennant; Cwm Glas area, Snowdon, 2008, D.J.T. (herb. **DJT**; **BM**)

In several cases not all of the specimens on the sheets for the above records were the same taxon; as few as a single specimen conformed to the Snowdonia *Cystopteris* in question in at least one case. A single specimen on the sheet which represents the 1853 record given above, and those representing the 1853 record are morphologically identical to the others, but require to be re-examined for the presence of glandular hairs. Glandular hairs were present on specimens representing the remaining seven records given above.

A specimen marked 'W.J. Hooker' in **BM**, but undated, was collected at Castell Dinas

Bran, Denbighshire, V.C.50, and named *Cyathea regia* by Hooker, but later renamed as a variety of *C. fragilis.* This record was listed by Tennant (2010), but is not the Snowdonia *Cystopteris* for which records are given above. This interesting specimen bears resemblance to those found in northern Scotland, which were mentioned in the Introduction, and require further investigation. A careful search by the author at Castell Dinas Bran and adjacent rocks failed to locate Hooker's fern, which is probably extinct there. Similarly, a recent but lesser search in Cwm Idwal did not reveal the Snowdonia *Cystopteris* taxon.

Following the discovery that the Snowdonia taxon was glandular, it was drawn to the attention of the author by another botanist, Brian Burrow, that *Flora Iberica* (Prada, 1986) describes an infra-specific form of *C. fragilis* that is distinguished by the presence of glandular hairs, namely subspecies *huteri* (Hausm. ex Milde) C. Prada & Salvo. The description, illustration and spore size given (44-55 (60) microns) for this subspecies matched those of the Snowdonia *Cystopteris* very well. Subsp. *huteri* is a taxon found mainly in montane regions of southern and western mainland Europe, with a general distribution similar to that of *C. alpina* (Lam.) Desv., and has been linked to the latter in Scandinavia (F.J. Rumsey, pers. comm.), although the extent of its further geographic limits is not certain.

In 2014 and 2015 fieldwork was carried out by the author in the central Italian Dolomites, which is the centre of distribution of subsp. *huteri* (F.J. Rumsey, pers. comm.). Subsp. *huteri* was found to be frequent there, enabling a comparison to be made with the Snowdonia *Cystopteris*, and material was donated to the NHM for further study by F.J. Rumsey. In the Dolomites *C. fragilis* subsp. *huteri* occurred mainly between 1600 and 2400m on dolomitic limestone in open, rocky, alpine woodland, extending to boulders and scree at the base of cliffs well above the tree-line, both habitats with a very rich alpine vascular flora. The other ferns often present were *Cystopteris fragilis* subsp. *fragilis, C. alpina, C. montana* (Lam.) Desv., *Gymnocarpium robertianum* (Hoffm.) Newman, *Asplenium trichomanes* L. subsp. *quadrivalens* D.E. Meyer emend. Lovis, *A. trichomanes-ramosum* L., *A. ruta-muraria* L. (including subsp. *dolomiticum*) and occasionally *Woodsia glabella* R. Br. ex Richards. *Cystopteris fragilis* subsp. *huteri* in the Dolomites was more variable than the Snowdonia fern, but close matches to the latter were found there, the majority being intermediate between *C. fragilis* and *C. alpina* in general appearance. F.J. Rumsey has agreed that these Snowdonia ferns should be referred to *C. fragilis* subsp. *huteri.*

A very small population of another morphologically distinctive *Cystopteris* was also found by the author in the Dolomites, very locally at 2300m, and this matched the fern mentioned in the Introduction that occurred in Scotland (Figures 3 & 4). The herbarium studies at RBGE (**E**) showed that this Scottish fern had been collected earlier there, and was re-found in Orkney, V.C. 111, in 2010, and later in 2014 by the author in West Ross, V.C. 105, then cultivated from spores. Morphologically similar ferns had also been seen by the author in the French Vanoise and Norwegian Alps. All of these ferns are somewhat intermediate in their morphology between *C. fragilis* and *C. alpina*. Those from mainland Europe occur there with *C. alpina* s. str., although often at lower altitudes, and at least some of these are currently referred to *C. alpina*. Living examples from both recently found localities in Scotland that resemble the ferns from mainland Europe have been donated to NHM for biosystematic analyses in order to study their phylogenetic relationships and to establish ploidy levels.

DISCUSSION

Various authors have commented on the difficulties of diagnosing taxa in the *Cystopteris fragilis* complex by use of morphological characters alone, for example Vida (1974) and Rothfels et al. (2013). Blasdell (1963), however, had successfully developed an evolutionary structure, which in most cases showed a good correlation with recent phylogenetic analyses (Rothfels et al., 2013). The main problems arise because of multiple ancestral origins, the existence of many polyploids, and in Europe, the absence of the ancestral diploid parent. In Europe *C. fragilis* is tetraploid, hexaploid, or octoploid, but in the British Isles the last is so far unknown. Intermediate ploidy levels have arisen through intraspecific hybridisation, and pentaploid examples are said to be frequent (Vida, 1974). It should therefore be emphasised that the main conclusion in this paper, namely the recognition of *C. fragilis* subsp. *huteri* in the British Isles, is very largely based on morphological characters. In this case, however, the morphology of this Snowdonia fern is quite distinct from other British variants of *C. fragilis*; the spores are exceptionally large, and the glandular condition is presently a unique character in European *Cystopteris*. These characters provide the confidence to refer the Snowdonia *Cystopteris* to this European taxon.

As stated above, diploid *Cystopteris* is not recorded in Europe, but in North America several glandular species of *Cystopteris* are known and their glandular condition has resulted from the donation of a maternal genome from the diploid plant, *C. bulbifera* (L.) Bernh. (Rothfels et al., 2003). One glandular North American allohexaploid species, *C. laurentiana* (Weatherby) Blasdell, which possibly arose as a cross between *C. fragilis* and *C. bulbifera*, has been suggested to be synonymous with *C. fragilis* subsp. *huteri* (F.J. Rumsey, pers. comm.), but that would imply that *C. bulbifera* at one time had occurred in Europe, which at present is not proven.

The Snowdonia fern here referred to *C. fragilis* subsp. *huteri* is clearly responsible for all of the previously published erroneous records in Wales for *C. alpina* s. str. (but see comments above in Results, and below regarding a Welsh fern named by W.J. Hooker). The Snowdonia fern does share some characters found in *C. alpina*, which may have led to the confusion, namely the close similarity of its juvenile sporophytes and the morphology of its immature pinnules (see under Results above and Tennant (2010)). These characters probably arose from an ancestral genome donated from *C. alpina,* which may therefore have once occurred in Wales. The very large spore size given for *C. fragilis* subsp. *huteri* in *Flora Iberica* was suggested by the authors of this publication to indicate that this taxon is probably octoploid. Spores of Snowdonia examples were found to be similar in size (F.J. Rumsey, pers. comm.), suggesting that these ferns were likely to be at least hexaploid, if not octoploid, a ploidy level not recorded for *C. fragilis* in the British Isles. Blasdell (1963) had suggested that spore size in *Cystopteris* could be used in the determination of the different cytotypes, but Vida (1974) found that, contrary to that opinion, spore size was not always reliable in this respect. R.H. Roberts measured the spores of two different examples of *C. alpina* for the author. These both had almost identical finely dissected fronds with linear pinnule segments that are associated with classic *C. alpina* s. str. (Figure 3), but were found to differ widely in spore size (Tennant, 1995), supporting Vida's findings, which require further investigation using the various cytotypes. Vida (1974) also found that a deliberately induced apogamous diploid of *C. fragilis* had a much more finely dissected frond than the natural tetraploid plants from which it had been derived, and that can clearly be seen in his Plate 2, fig. 9, which might suggest that degree of frond dissection may not necessarily increase with an increasing

ploidy level.

Historic *Cystopteris* nomenclature used by British authors is very relevant to *C. fragilis* subsp. *huteri* from Snowdonia. This particularly applies to the names *'incisa'*, *'regia'* and '*alpina'*, and is also relevant to the related taxa from Scotland and other taxa from Wales, which are discussed earlier in this paper. Smith (1794) in Volume 3, p.163 published the name *Cyathea incisa* Sm., 'Laciniated Cup-fern' when describing and illustrating a fern collected pre 1790 by E. Forster at a single locality near Walthamstow in South Essex, V.C. 18. Smith stated that this fern was not the *Polypodium regium* of Linnaeus and was also different from *P. alpinum* Jacquin. Smith's description of this fern gives 'frond bipinnate', (the pinnule lobes) 'obtuse with no bristly point', the last character clearly seeming to distinguish it from the Snowdonia subsp. *huteri.* Smith (1828; 1834) later published the name *Cystea regia* Sm., and here he cited Lhwyd's late 17th century Snowdon record, two further records from Snowdon, namely one by the Rev. Hugh Davies in the early 19th century and one by W. Wilson in 1826, and a further record by Mr. Griffith from Cwm Idwell (Idwal), which all almost certainly referred to the Snowdonia subsp. *huteri.* Smith, however, surprisingly also added here the Essex fern (*Cyathea incisa* and *C. regia* Forster), thus seeming to suggest that these were synonymous with the Snowdonia ferns, even though their descriptions differed. Smith also stated that *Cystea regia* Sm. was unquestionably distinct from every other British fern, and from the *'regia'* of alpine stations (in Europe), presumably meaning the fern now referred to *Cystopteris alpina* (Lam.) Desv., although confusingly he included *Aspidium regium* Sw. (1802) in the synonymy.

W.J. Hooker (1842) then grouped together all of Smith's taxa under *C. alpina* Desv., and added 'tripinnate' to Smith's earlier 'bipinnate' description of these taxa. Hooker had earlier (before 1842) confirmed the name of one of the specimens from Essex collected by Forster as *Cyathea regia* (Stansfield, 1930), and in the same period had also given the same name to an undated specimen in **BM** that had been collected in Denbighshire. V.C. 50. These Essex and Denbighshire records, in the sense of Hooker (1842), therefore represent the first and second British records respectively for *C. alpina.* J. Backhouse's record from Upper Teesdale in Yorkshire, V.C. 65 in 1872 (Tennant, 2010) would therefore be the third British record. In spite of Smith (1828; 1834) and Hooker (1842) including the Snowdonia ferns under the species which is now referred to *C. alpina* (Lam.) Desv., *C. fragilis* subsp. *huteri* from Snowdonia is shown here to be different, and its first name, *Cystea regia* sensu Sm. (parte) should be retained only in synonymy.

The use of the name *'regia'* for some of these ferns is, however, incorrect on the assumption that the name *'regia'* was entirely derived from *P. regium* L. The type sheet of *P. regium* in **LINN** contains more than one species and *P. regium* was therefore a 'nomen ambiguum'. None of the species on this sheet represent the taxa discussed in this paper, including *C. alpina* (Lam.) Desv.. This has long been known (Stansfield, 1929), and much earlier, for example *P. regium* L. was included by J.T. Syme in the synonymy under *C. fragilis* in the third edition of *English Botany*, Vol. 12 (1886), which would be correct, although an individual specimen still requires to be selected from the type sheet and formally lectotypified as such.

Other authors also considered '*regia*' to be distinct. Milde made '*regia*' a variety of *C. alpina* (Kestner, 1930), and Stansfield (1929) proposed the name *C. alpina* var. *obtusa* for Forster's *Cystopteris* from Essex. After examining the Essex specimen, Stansfield pointed out that it was bipinnate, with only shallowly notched pinnule lobes, many of

the veins terminating in an apical point, unlike *C. alpina* s.str., which is at least tripinnate with virtually all the veins ending in notches. Kestner (1930) had stated that the position of the vein-endings was the only reliable character in the determination of *C. alpina,* additionally the *Cystopteris* key given in Murphy and Rumsey (2005) similarly relies on this character for the determination of *C. alpina*.

The first name given to the Essex fern, *Cyathea incisa* Sm. (1794), would be a legitimate name at specific level, and for the Denbighshire *Cystopteris* as this appears to belong to the same taxon. It is doubtful, however, whether these ferns could be justified as a species distinct from *C. alpina*, in which case the name would be reduced to synonymy under the latter. The Essex and Denbighshire ferns are both thought to be extinct at their original localities. A similar Welsh taxon from Merionethshire, V.C. 48, has not been collected since 1928, so none of these ferns are presently available for biosystematic studies that might elucidate their correct status. The *Cystopteris* taxon from Scotland discussed briefly above also seems to have affinities with *C. alpina*. Two examples of this and a morphologically identical fern from Italy are in cultivation by the author, and material is now at NHM for further studies to establish their status.

Several recently confirmed specimens of *C. alpina* seen by the author in the Tromsø Museum herbarium had exceptionally broad pinnule lobes. Following the fieldwork by the author, uniformly similar *Cystopteris* forms were also encountered in alpine regions of France, Italy, and in Scotland, some of which are presently determined as *C. alpina* (Lam.) Desv. This might suggest that the concept of *C. alpina* s.str. as a fern restricted to one with a finely dissected frond and linear pinnule lobes, and with the veins virtually all ending in notches, unlike the *'regia'* of some authors, may be one that is too narrow. An alternative suggestion may be that these ferns with broader pinnules truly represent a distinct taxon, which is affiliated to *C. alpina* through the presence of a genome from this species. Hopefully biosystematics will resolve this.

Cystopteris fragilis subsp. *huteri* is presently known in Britain only from two localities in North Wales, where it is very local and scarce. It meets the I.U.C.N. Threat Category of Critically Endangered (CR) and should be considered for inclusion in the British Red Data List, its main vulnerability being one of collecting.

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